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NITROGEN-FIXING BACTERIA OF THE GENUS *BEIJERINCKIA* IN SOUTH AFRICAN SOILS

by J. H. BECKING

Laboratory of Microbiology, Agricultural University, Wageningen, the Netherlands

INTRODUCTION

In 1939 Starkey and De [23] described a new *Azotobacter* species, *Azotobacter indicum* isolated from an acid rice soil in India, which is capable of fixing atmospheric nitrogen under acid conditions [21 22].

In 1950 Derx [2 3] found this microbe to occur abundantly in some Bogor (W. Java) soils and proposed the new generic name of *Beijerinckia* for it in view of differences in morphology and cultural characteristics that distinguish the latter from the other *Azotobacter* species.

In recent reviews [9 14 26 27] on nitrogen-fixing bacteria the setting up of a new genus *Beijerinckia* for *Azotobacter indicum* seems to be accepted.

Unlike the almost ubiquitous occurrence of *Azotobacter* which has been encountered in all soils with suitable pH independent of their geographical localization, the distribution of *Beijerinckia* is restricted to tropical regions. There exists no definite explanation for this phenomenon.

In addition to the localities mentioned above, *Beijerinckia* has been reported from Malaya (Altson [1]), French West Africa (Kauffmann and Toussaint [11 12]), Madagascar (Dommergues [6]), Australia north of the 20°S. latitude (Tchan [28]), and South America (Döbereiner and De Castro [5], Kluyver and Becking [13]). Meiklejohn [15] reported the isolation of a green fluorescent pigment-producing *Beijerinckia* strain from an acid Tanganyika soil, but later she identified this micro-organism as belonging to *Azotobacter vinelandii* Lipman [16].

Unpublished results obtained by the author indicate the occurrence of *Beijerinckia* in lateritic soils or latosols from the Hawaii Islands, New Guinea (Papua), China (Kwantung province, Hongkong), Ethiopia, East Africa (Tanganyika, Uganda, Kenya) and in some regions of South America (Trinidad Island, Dutch Guiana, Brazil, and Bolivia).

Again all these localities for *Beijerinckia* are tropical, although some of them are at rather high altitudes *i.e.* at 7,000 to 9,000 feet in the Ethiopian and East African highlands.

There is only one record of the occurrence of *Beijerinckia* outside the tropics. In a very recent publication Suto [24 25] describes the isolation of this bacterium from an acid volcanic ash soil at Sendai (latitude 38° N.) in Japan.

The present paper deals with the unsuspected occurrence of *Beijerinckia* in South African soils. South Africa lies outside the tropical bounderies between latitudes 23° and 34° S. This fact is of considerable importance since Tchan [28] has stated explicitly that *Beijerinckia* does not occur in the Australian continent at latitudes more south than 20° S.

MATERIALS

The soils were sampled in the field in small, sterile containers at their natural moisture content. The samples were all surface soils. They were not a random sample. Most of them were collected in forest plantations and along roadsides. Soil pH was measured by a glass electrode in the soil paste obtained by stirring some soil with a small amount of distilled water.

The time between soil sampling and microbiological examination was about 3–6 weeks. It is assumed that this delay has been of no influence on the results as *Beijerinckia* appears to be very persistent in soils in which it naturally occurs. Tropical latosols stored for five years in an air-dry condition at room temperature still reveal living *Beijerinckia* germs on examination.

A geographical map (Fig. 1) gives the distribution of the soil samples over the Union of South Africa. In the list of soil samples (Table 1) some details concerning the various samples are given such as locality, latitude, annual rainfall, altitude, occurrence of frost, soil type, soil pH, and cover. Most of these data are derived from the publications on South African soils and forest trees of Van der Merwe [17] and Poynton [19]. Geological information was obtained from the geological map (scale 1 : 1,000,000) of the Department of Mines, Geological Survey 1955, Pretoria.

METHOD OF EXAMINATION

The predominant characteristic of the bacteria of the genus *Beijerinckia* is their acid tolerance. Growth is obtained between pH = 3.0 and pH = 10.0. Their development is most vigorous at a pH = 4.0 to 5.0, which is usually about the reaction of their natural surroundings. However, growth of *Beijerinckia* in general is much slower than that of *Azotobacter*.

For the reasons mentioned above and in order to eliminate *Azotobacter*, an acid enrichment medium was employed. This medium differs only from the usual enrichment medium of *Azotobacter* by its acidity of pH = 5.0.

The composition of the medium used is quite simple:

Distilled water	1000 ml	KH_2PO_4	1.0 g
Glucose	20.0 g	$MgSO_4.7H_2O$	0.5 g

The phosphate buffer gives a pH of about 5.0. The supply of trace elements in the medium was obviously provided by the soil used as inoculum. Supplementary trace-element addition yielded no better results especially as fungus growth was enhanced.

The enrichment medium was poured in very thin layers (2 to 3 mm) in petri dishes (9 cm diameter) to ensure adequate aeration and to reduce butyric-acid fermentation. Petri dishes as well as enrichment media were sterilized before use in order to eliminate *Beijerinckia* infections from other sources than the soil used as inoculum.

For inoculation approximately 0.1 to 0.5 g of soil per petri dish was used. Every soil was tested with 10 to 15 replicates.

The enrichment cultures were examined microscopically for the presence of *Beijerinckia* cells which owing to their peculiar morphological characteristics can easily be recognized. *Beijerinckia* cells are motile or non-motile gram-negative rods (approx. 1.2 to 4.8–7.2 μ) showing a large lipoid globule stainable with Sudan III at each end of the cells. (See Plate 1A). Some strains produce large capsules with a distinct wall layer enveloping a large number of cells (see Plate 1B).

If necessary, the microscopic examination of the enrichment cultures was extended over a longer period of 3 to 4 weeks. A positive result on the basis of microscopic examination was always

checked by streaking an appropriate dilution of the enrichment medium on plates containing 2% agar, 2% glucose, 0.1% KH_2PO_4, 0.05% $MgSO_4.7H_2O$, 0.01% $FeCl_3.6H_2O$, 0.002% $Na_2MoO_4.2H_2O$ (pH = 5.0). Calcium was not added to the medium as *Beijerinckia* needs no calcium for development. On this medium *Beijerinckia* forms highly raised, often plicated, glistening colonies of a very tenacious and elastic slime. The copious production of slime is a distinct feature of the genus (see Plate 2).

As a rule the presence of *Beijerinckia* cells in the enrichment medium can already be established with certainty by judging the appearance of the medium. In a somewhat advanced stage of development the medium changes into a viscous slimy mass which can be drawn out by a loop in long threads. The yeast, *Lipomyces* species, often present in these nitrogen-poor enrichment cultures, never produces such a tenacious slime.

RESULTS

Table 1 shows that most of the South African soils tested are distinctly acid. Only five of the forty samples collected showed pH values above pH = 6.0, *viz* a calcareous littoral soil (pH = 7.7) from Port Durnford, an alluvial (Olifant's river C.P.) desert soil from the Little Karroo, a savannah soil (pH = 6.7) from Pretoriuskop (Krüger National Park), a dressed (limed?) citrus soil (pH = 6.6) from Nelspruit, and a garden soil (pH = 6.1) from the Tokai Arboretum near Muizenberg, Cape Peninsula.

Twelve of the thirty-five more acid soils (pH $\leqslant$ 6.0) and two

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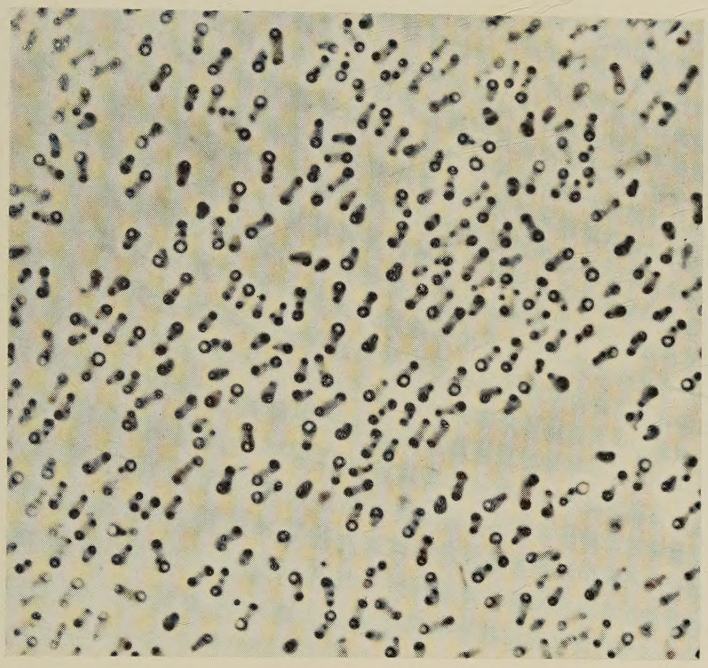
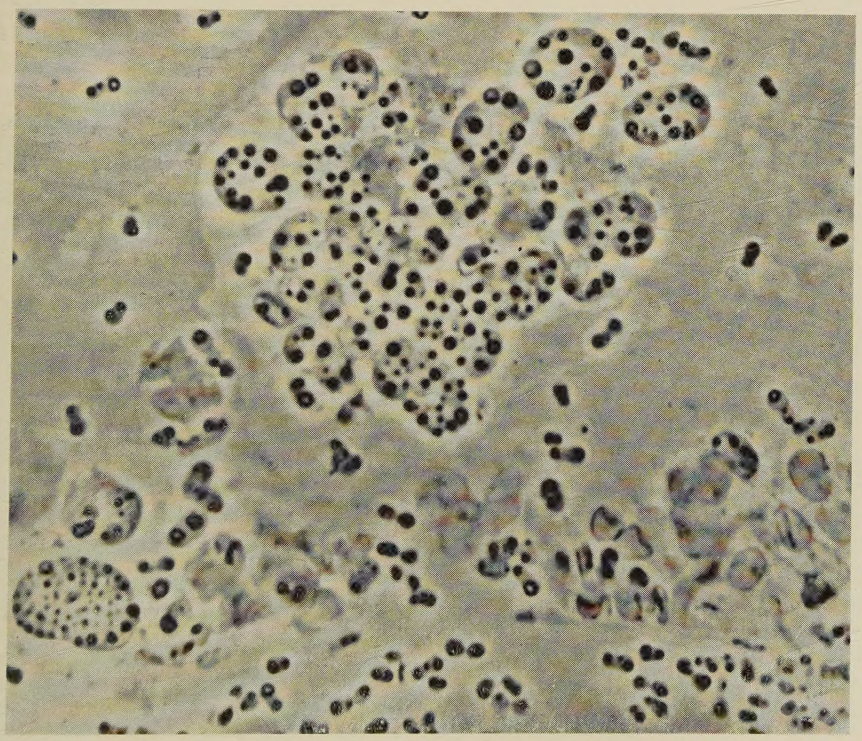
Plate 1: Both pictures are phase-contrast photographs of living cells grown on nitrogen-free glucose agar (pH = 5.0) at 30° C. (Magnification 2000 ×).

A. Cells of a *Beijerinckia* strain isolated from a South African "Mistbelt" soil. The photograph shows typical *Beijerincka* cells characterized by a large, highly refractive fat globule at each end of the cell.

B. *Beijerinckia* strain isolated from South African (Tweefontein plantation, Sabie) soil of pH = 4.5.
This strain shows the property of producing large capsules with a distinct wall layer enclosing many cells.
Similar strains were isolated from some other South African soils (*e.g.* Amatikulu soil) and from other regions (China, Java, Brazil).

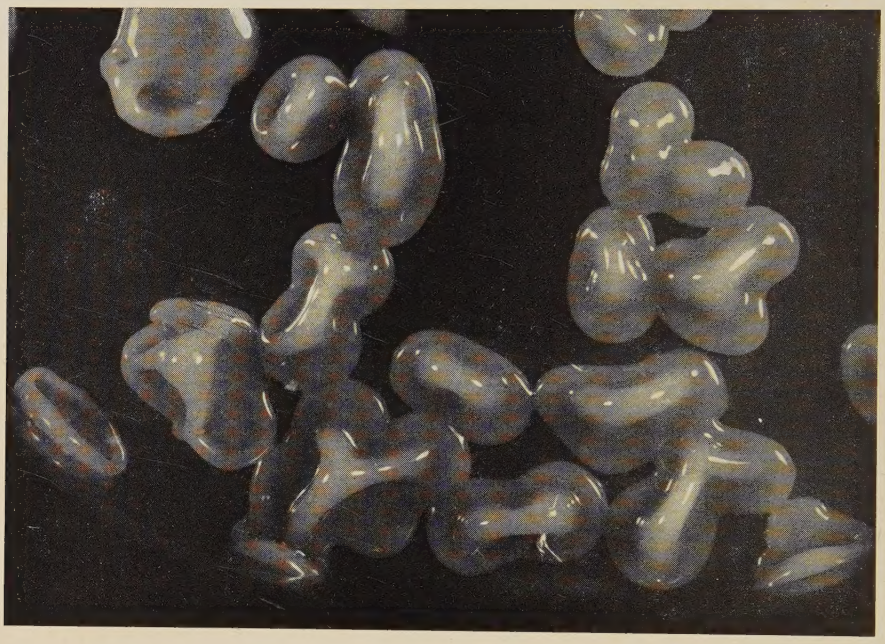


A



B

Plate 1



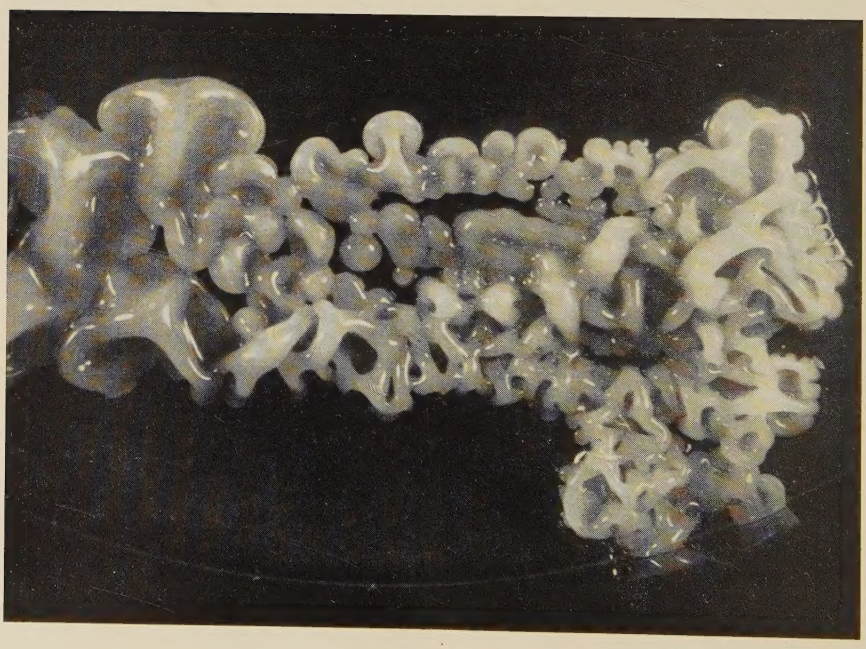


Plate 2

of the five soils with pH values higher than pH = 6.0 showed *Beijerinckia* development. As evident from Table 1 *Beijerinckia* was absent in the very acid soils (pH < 4.3) and the alkaline soils (pH > 7.0).

As indicated in the geographical map (Fig. 1) eleven of the twenty soils collected around or north of latitude 29° S. yielded *Beijerinckia*, but only three of an equal number of soils collected south of this latitude. This confirms to a certain extent the tropical distribution of the genus *Beijerinckia*.

Beijerinckia was found to be present abundantly in the northern part of Transvaal in the soils (pH = 5.8 to 6.1) of the De Hoek plantations, north of Tzaneen in the Drakensberg Mountains, in the soils (pH = 5.3) of Wilgenboom plantation, north of Acornhoek, and in the soils around Sabie at Tweefontein plantation, Witklip plantation and the S.A.F.I. estate (Hendriksdal) with pH values of 4.5, 5.4 and 5.8, respectively.

It occurred with *Azotobacter* in the savannah soil (pH = 6.7) from Pretoriuskop in the Krüger National Park. Moreover, *Beijerinckia* was present in considerable numbers in the acid soil (pH = = 4.5) of the Usutu pine plantations near Mbabane in Swaziland.

In Natal the acid sands (pH = 4.8) of Kwambonambi, St. Lucia Bay and the acid coastal soils near Eshowe, *viz* those near Port Durnford (pH = 4.5) and Amatikulu (pH = 5.9), contained some *Beijerinckia* germs.

Beijerinckia was found to be distributed far more sporadically in soil samples collected in the Cape Province. Only in three cases a positive result was obtained, *i.e.* in a forest soil (pH = 4.4) of the Forest Sanctuary near Stutterheim, in a well-developed *Pinus taeda* forest soil (pH = 5.4) with root penetration to 2 meters (very good site) at Blueliliesbush, and in a garden soil (pH = 6.1) of the Tokai Arboretum near Muizenberg on the Cape Peninsula. In the latter soil *Beijerinckia* occurred together with *Azotobacter*.

Soil cover seems of little influence on the distribution of *Beije-*

Plate 2: Typical colony types of *Beijerinckia* strains.

Most strains produce these highly raised, often plicated, glistening colonies of a very tenacious and elastic slime.

Colony development upon nitrogen-free glucose agar plates (pH = 5.0) incubated 3 weeks at 30° C. (Magnification 2 ×).

rinckia as the latter occurs in soils under indigenous grass cover (North Transvaal), savannah vegetation (Low Veld), sugar-cane cultivations (Amatikulu), exotic forest plantations (Transvaal, Swaziland, Natal), natural forest (Stutterheim Forest Sanctuary) or garden soil (Tokai Arboretum).

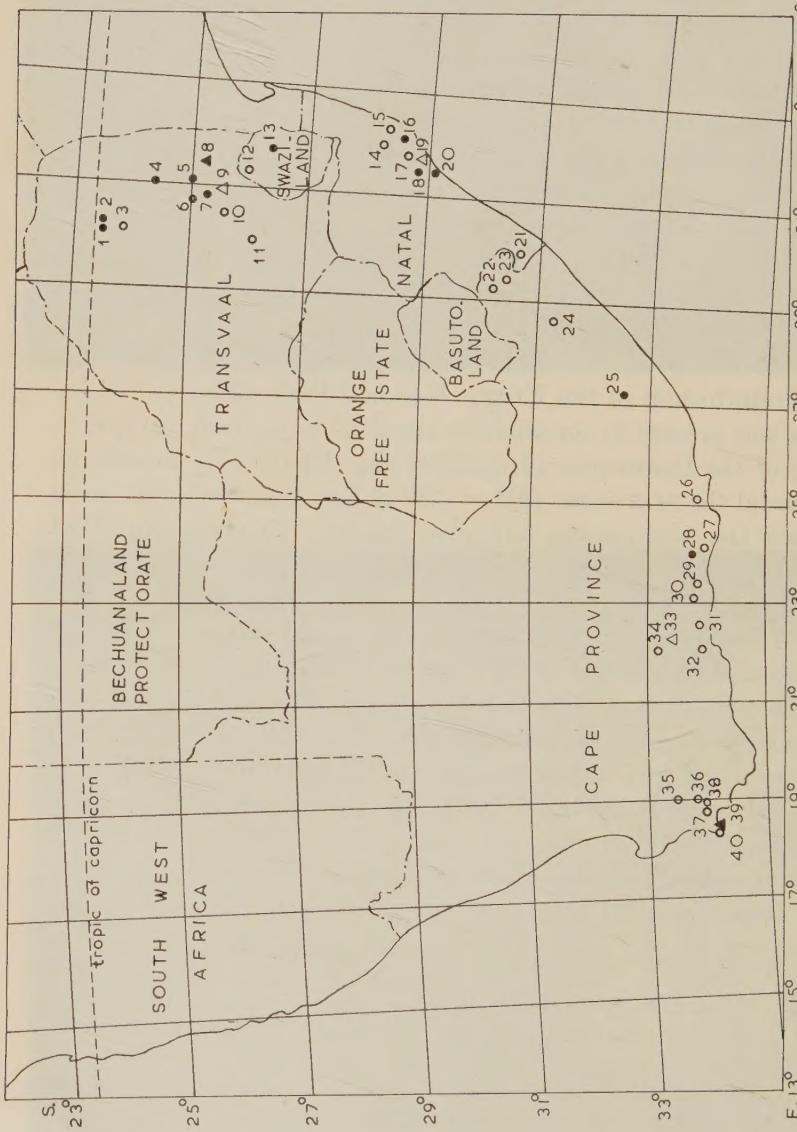


Fig. 1. Geographical distribution of the soil samples collected in the Union of South Africa. Notation: negative samples (○), soils containing *Beijerinckia* (●), *Azotobacter* (△) and both species (▲). The numbers correspond to the soil sample numbers presented in Table 1.

Climate – apart of its influence on soil type – also shows little influence. In northern Transvaal *Beijerinckia* occurs predominantly in the high-rainfall (40–66″) area in the Drakensberg Mountains, sometimes at rather high altitudes (3,000–6,000′) where occasional frosts occur. But this micro-organism is also present in the semi-arid savannah climate of the Low Veld with extremely hot day temperatures and in soils of the Tokai Arboretum with a moderate climate.

In contrast to the factors mentioned, soil type appears to have very great influence on the geographical and ecological distribution of *Beijerinckia*. This will be discussed more in detail in the next section.

DISCUSSION

The ecological and geographical conditions governing the remarkable tropical distribution of the genus *Beijerinckia* are not clearly understood.

In view of the fact that these micro-organisms have been usually reported from more or less acid soils, one may surmise that a low pH, by suppressing the competition of many other soil bacteria, might be a factor determining the presence of *Beijerinckia*. The negative outcome of tests with so many and diverse acid soils of temperate regions shows the untenability of this idea.

There is also no reason for the belief that the low temperature in the temperate zones may be responsible for the absence of *Beijerinckia* in these soils. Growth experiments with *Beijerinckia* strains at several temperatures showed no marked differences between temperature optima and minima of these bacteria and those of *Azotobacter* strains. In fact, the reverse was found to be true of what would have been expected from a tropical micro-organism as no *Beijerinckia* strain was found to produce growth at 37° C while most *Azotobacter* strains grew profusely at this temperature. Some *Azotobacter* strains still produced growth at 40° C. These experiments will be described in a subsequent paper.

Moreover, *Beijerinckia* is fairly cold and frost resistant. Cells of these micro-organisms suspended in tap water or nitrogen-free mineral medium and stored for twenty months at + 4° C or — 4° C in a refrigerator retained some viability as shown by growth when

TABLE 1

Sample No.	Locality	Lat. °S.	Annual rainfall, inches	Altitude Ft.	Sam No
	TRANSVAAL				
1	De Hoek plantations, 7.5 miles N. of Mokeetsi, N. of Tzaneen	23.5	66	4000	
2	„ „ „ 7.5 „ „ „ „ „ „ „ „ , Bruynzeel's Sawmill	23.5	66	4000	
3	Westfalia estate, between Duivelskloof and Tzaneen	24.0	40	2800	
4	Wilgenboom plantation, 12 miles NW. of Acornhoek	24.5	50	3000	
5	Tweefontein plantation, road between Graskop and Sabie	25.0	48	4100	
6	Witklip plantation, SE. of Sabie	25.0	45	3800	
7	South African Forest Investment estate, Hendriksdal, 7 miles S. of Sabie	25.0	45	4400	
8	Krüger National Park, Pretoriuskop	25.0	22	1060	
9	Citrus and Subtropical Horticulture Research Station, Nelspruit	25.5	30	2350	
10	Berlin plantation, 20 miles SW. of Nelspruit	25.5	42	4400	1
11	Jessievale plantation, 25 miles N. of Ermelo	26.0	36	5800	1
	SWAZILAND				
12	Piggspeak plantations, 10 miles N. of Piggspeak	26.0	47	4500	1
13	Usutu plantation, 30 miles NE. of Mbabane	26.0	45	4500	1
	NATAL				
14	Dududuku plantation, 10 miles NW. of St. Lucia	28.0	38	200	1
15	Vergoeding plantation, 8 miles NW. of St. Lucia	28.0	38	200	1
16	Kwambonambi, 14 miles NE. of Empangeni	28.5	50	100	1
17	5 miles N. of Empangeni	28.5	45	200	1
18	Port Durnford, 20 miles E. of Eshowe	29.0	60	300	1
19	„ „ 20 „ „ „ „	29.0	60	50	1
20	Amatikulu, 20 miles SE. of Eshowe	29.0	36	100	2
21	Weza plantations, 30 miles E. of Kokstad	30.5	45	4500	2
	CAPE PROVINCE				
22	Houtbosfontein plantation, 20 miles N. of Kokstad	30.5	45	5000	2
23	„ „ , 15 miles N. of Kokstad	30.5	45	3000	2
24	Arboretum Nqadu, 15 miles NW. of Umtata	31.5	39	2900	2
25	Forest Sanctuary, 20 miles W. of Stutterheim	32.5	42	2500	2
26	Loerie plantations, 30 miles SW. of Uitenhage	34.0	31	2400	2
27	Witelsbos, 60 miles W. of Humansdorp	34.0	45	700	2
28	Blueliliesbush, 80 miles W. of Humansdorp	34.0	40	700	2
29	Kransbos, 10 miles NE. of Knysna	34.0	31	1200	2
30	Concordia plantations, 10 miles N. of Knysna	34.0	38	800	3
31	Saasfeld, 15 miles E. of George	34.0	34	600	3
32	Kleinplaat, 20 miles W. of George	34.0	22	2000	3
33	Little Karroo, Olifant's river, 10 miles N. of Oudtshoorn	33.5	10	1100	3
34	Swartberg Pass, 10 miles S. of Prince Albert	33.5	18	3000	3
35	Kluitjeskraal, 1 mile W. of Wolseley	33.5	26	900	3
36	Franschhoek, Helshoogte, 5 miles NE. of Stellenbosch	34.0	29	1000	3
37	Jonkershoek, 10 miles SE. of Stellenbosch	34.0	42	900	3
38	„ 10 „ „ „ „	34.0	42	900	3
39	Tokai Arboretum, Muizenberg	34.0	38	200	3
40	Hout Bay, 10 miles S. of Cape Town	34.0	25	100	4

plated on nitrogen-free nutrient agar. Such extreme conditions are not likely to occur in temperate zones. Ecological evidence also points to a considerable cold tolerance of *Beijerinckia* as it was found to occur at rather high altitudes in the Red Earths of the

TABLE 1 (continued)

Frost	Soil type	Soil pH	Soil cover	B*	A**	Sample No.
ght	Red lateritic loam	5.8	*Eucalyptus microcorys* cultivation	+	–	1
,,	,, ,, ,,	6.0	Indigenous grassland	+	–	2
,,	,, ,, ,,	5.0	*Eucalyptus saligna* cultivation	–	–	3
,,	,, ,, ,,	5.3	*E. cloeziana* cultivation	+	–	4
derate	,, ,, ,,	4.5	*E. saligna* cultivation, 9 years old	+	–	5
ght	,, ,, ,,	5.4	*Pinus patula* cultivation	+	–	6
,,	,, ,, ,,	5.8	*P. patula* cultivation, 35 years old	+	–	7
ne	Reddish-brown sandy soil	6.7	Savannah vegetation	+	+	8
,,	,, ,, ,,	6.6	*Citrus* cultivation	–	+	9
derate	Yellow-brown sandy soil	4.2	*Pinus patula* cultivation, old plantation	–	–	10
vere	Red lateritic loam	4.6	*P. patula* cultivation, young plantation	–	–	11
d	Red lateritic earth	5.2	*Pinus patula* cultivation	–	–	12
	,, ,, ,,	4.5	*P. patula* cultivation, 6 years old	+	–	13
ne	Light brown littoral sand	4.4	*Pinus caribaea* cultivation, 8 years old	–	–	14
	,, ,, ,, ,,	4.5	*Eucalyptus paniculata* cult., 3–4 years old	–	–	15
	Brown fine sand	4.8	*Pinus elliottii* cultivation	+	–	16
	Humus-rich forest soil	5.6	*Casuarina equisetifolia* cultivation	–	–	17
	Reddish-brown fine sand	4.5	*Eucalyptus maculata* cult., 30 years old	+	–	18
	Dark littoral sand	7.7	*Casuarina equisetifolia* reafforestation	–	+	19
	Yellow-brown ferruginous earth	5.9	Sugar-cane cultivation	+	–	20
derate	Yellow-brown lateritic loam	4.5	*Pinus patula* cultivation, 31 years old	+	–	21
,,	Red (eluvial) dolorite soil, slopes	5.7	Natural pasture	–	–	22
,,	Dark (illuvial) soil, valleys	5.4	Uncultivated	–	–	23
,,	Reddish ferruginous earth	4.3	Arboretum	–	–	24
ht	Red lateritic loam	4.4	Bare slope	+	–	25
	Gray fine loam	5.1	*Pinus pinaster* cultivation	–	–	26
	,, ,, ,,	5.1	*P. pinaster* and *P. radiata* cult., poor site	–	–	27
	,, ,, ,,	5.4	*P. taeda* cult., 41 years old, very good site, root development to 2 m	+	–	28
	,, ,, ,,	4.4	*P. taeda* cultivation	–	–	29
ne	Dark sand (old dunes)	3.6	,, ,, ,,	–	–	30
ht	Grey ferruginous sand	5.9	Natural forest (groenbos)	–	–	31
	Dark sandy soil	3.6	Forest felling area	–	–	32
ere	Heavy loam (riverside)	7.5	Cultivated soil	–	+	33
	Brown sand	4.7	Reafforestation of eroded land	–	–	34
ht	Iron-coated coarse sand	4.9	*Pinus taeda* cultivation	–	–	35
	Yellow-gray sandy soil	5.1	,, ,, ,,	–	–	36
	Dark litter-rich sandy soil	5.5	*Pinus radiata* cultivation	–	–	37
	Reddish-gray fine sand	5.1	Natural forest (fijnbos)	–	–	38
ne	Garden soil	6.1	Arboretum	+	+	39
	Reddish coarse granitic sand	5.1	Indigenous vegetation	–	–	40

resence of *Beijerinckia*. **A = presence of *Azotobacter*.

Ethiopian Mountain Plateau (Addis Ababa and Entoto hills at 8,300 and 9,300′, respectively), in the latosols of the East African Highlands (Kenya, 6,800′) and of the Drakensberg Mountains (4,000′) in South Africa, and in some mountain forest soils (6,700′

to 10,000') in Java. Several of these localities suffer occasional frosts.

We must therefore conclude that the occurrence of *Beijerinckia* is determined by some complex of ecological factors which is only realized in the tropics.

Several hypotheses have been developed to explain the tropical distribution of *Beijerinckia*.

Derx [4] attributes the tropical distribution to a possible association with some non nodule-forming tropical legumes of the genus *Caesalpiniaceae* (e.g. *Cassia tora*) and suggests that *Beijerinckia* is a facultative symbiont which in contrast to *Rhizobium* has not lost the power to fix atmospheric nitrogen outside the plant.

Ruinen [20] reports the abundant occurrence of *Beijerinckia* on leaves ("phyllosphere") of tropical plants. She is inclined to think that there exists in tropical regions a mutual benificial relationship between tropical plants and *Beijerinckia*. The plant would provide the bacterium through cuticular excretion with sugar and nutrient salts, whilst the bacterium would fix atmospheric nitrogen which might become available to the plant.

Kluyver and Becking [13] have drawn attention to the fact that in nearly all cases in which *Beijerinckia* has been isolated from soil, a tropical soil of the eluvial type, *i.e.* a lateritic soil (*e.g.* laterite, tropical Red Earth or latosol) is concerned. *Beijerinckia* is observed to be absent in tropical illuvial soils (*e.g.* calcareous Margalitic soils, Black Cotton soils, Regur or Mbuga soils) and in tropical alkaline alluvial (river or marine deposits) clay soils.

They suggest that the leached lateritic soils are a favourable habitat for *Beijerinckia*. If we accept the view that the various *Beijerinckia* species are especially adapted to lateritic soils, we have to a certain extent an explanation of their restriction to the tropics as this type of rock weathering and clay mineral synthesis is only clearly displayed in the tropics.

From the geographical map (Fig. 1) it is evident that most *Beijerinckia* positive localities are situated between longitude 29° and 32° E. and latitude 23° and 29° S. This region comprises the Drakensberg Mountains forming a wall-like barrier from south to north extending form the SE. point of the Basuto Highlands, along the western boundery of Natal and the eastern side of Swaziland (Mbabane) into Transvaal past Pilgrims Rest, Tzaneen to SE. of Louis Trichardt in Northern Transvaal.

The escarpment of the Drakenberg Mountain rises abruptly from the Low Veld forming a transitional belt between the Low Veld and the High Veld north of Basutoland and the Low Veld and the Middle Veld in the

north. These mountain barriers, towering from 2,000 to 5,000 feet above the Low Veld, intercept the moisture-laden winds from the Indian Ocean, causing an increased precipitation on its eastern slope. This area – generally known as the "Mistbelt" – experiences the highest rainfall and the most humid atmosphere of the whole of South Africa.

Van der Merwe [17] (p. 182), in the publication "Soil groups and subgroups of South Africa", remarks: "Coincident with the "Mistbelt" there occur soil zones unique in South Africa. Although these zones differ from each other morphologically and chemically, they are classified as (a) Laterites, (b) Lateritic Red Earths and (c) Lateritic Yellow Earths". He classifies the soils near Sabie and Graskop (soil samples No's 5, 6, 7) as belonging to laterites, those near Tzaneen (soil samples No's 1, 2) and Mbabane (soil sample No. 13) as lateritic Red Earths, and those of Jessievale (soil sample No. 11) as lateritic Yellow Earth.

According to the geological map of the Geological Survey 1955, these lateritic soils of the "Mistbelt" are mainly derived from acid igneous rock, Old Granite of archaeozoic origin.

The remarkable coincidence given above between the distribution of *Beijerinckia* and the presence of laterites and lateritic Earths in South Africa strengthens our suggestion that *Beijerinckia* is mainly associated with lateritic soil types. Moreover it provides the first evidence of an earlier prediction of ours [13] that this micro-organism may occur also in lateritic soils outside the tropics.

The other soils observed to be positive for *Beijerinckia* are more or less ferruginous acid forest soils, near Stutterheim and Blue-liliesbush. The presence of *Beijerinckia* in a garden soil of the Tokai Arboretum on the Cape Peninsula indicates its occurrence in South Africa as far south as Cape Town lat. 34° S.

The abundant distribution of *Beijerinckia* in the latosols of the "Mistbelt" may be of some value in the nitrogen economy of these soil types. The pH values of these soils are mostly fairly low so that *Azotobacter* is absent.

As is well known, laterization leads to an accumulation of iron and aluminium in the upper soil layers and to a leaching away of silica, potassium, sodium and magnesium and more particularly calcium. The soils are moreover mostly phosphorus deficient owing to phosphate fixation by iron-aluminium complexes. Judged by the high electrical resistance, these soils contain very little soluble salts. The soils have a characteristic low base-exchange capacity due to the dominant presence of the clay mineral kaolinite.

It is tempting to relate the extreme poverty in calcium of the

natural surroundings of *Beijerinckia* with Jensen's [7 8] observation that these bacteria, in contrast to *Azotobacter*, require no calcium for their development. This observation has been confirmed by the present author. According to a recent publication by Norris and Jensen [18], however, calcium is not essential for the growth of *Azotobacter agile* either, although they found it to have a more or less stimulatory effect.

In these lateritic soils the accumulation of sesquioxides of iron and aluminium combined with a high acidity leads to a rather high content of mobile iron and aluminium and a low concentration of phosphate in the soil environment. The occurrence of *Beijerinckia* under these particular chemical soil conditions corresponds with our observations that *Beijerinckia* requires far more iron and less phosphate for optimal development than *Azotobacter*. Moreover, Katznelson [10] has shown that *Beijerinckia* (=*Azotobacter indicum*) is considerably less susceptible to the toxic effect of free aluminium than *Azotobacter* strains. A more detailed account on the mineral nutrition of *Beijerinckia* will be published in a separate paper.

SUMMARY

In contrast to the ubiquitous distribution of the nitrogen-fixing bacteria of the genus *Azotobacter* which can be encountered in all soils with suitable pH, the nitrogen-fixing bacteria of the genus *Beijerinckia* seem to be restricted to the tropics.

All attempts to isolate *Beijerinckia* from north-european soils have so far failed. The conditions underlying this peculiar phenomenon are not clearly understood. The selective action of low soil pH, low temperature, frost resistance, etc. cannot be responsible for the absence of *Beijerinckia* from temperate zones.

In an earlier paper [13] it was emphasized that the presence of *Beijerinckia* is almost invariably connected with the occurrence of laterites, tropical Red Earths or latosols. As these soil types are almost restricted to the tropics, this may be the reason why the genus *Beijerinckia* is mainly confined to tropical regions.

It was also suggested in that paper that *Beijerinckia* might also occur in lateritic soils – fossil or developed under particular climatic conditions – outside the tropics.

The present paper gives the first evidence of the occurrence of *Beijerinckia* in lateritic soils outside the tropics in South Africa. There *Beijerinckia* occurs abundantly in the lateritic soils of the "Mistbelt", but it occurs also – although more sporadically – in soils as far south as Cape Town (lat. 34° S.).

Hence, non-symbiotic nitrogen fixation may occur in some of these soils which are too acid to contain *Azotobacter*.

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GROWTH INHIBITION OF *CHLORELLA PYRENOIDOSA* PRODUCED BY SODIUM DIHYDROGEN PHOSPHATE AND ITS REVERSAL BY CALCIUM

by CLYDE EYSTER

Charles F. Kettering Foundation, Yellow Springs, Ohio

INTRODUCTION

Eyster [4] formulated a special medium for *Chlorella* based on the critical concentrations of nutrients for autotrophic growth [5]. The critical concentration of a nutrient element is defined as the minimum amount of the element which will produce maximum growth. The special medium proved to be seriously inadequate producing only 25 per cent of the growth usually obtained in the regular Warburg and Burk medium [19]. Separate additions of magnesium sulphate, potassium dihydrogen phosphate and sodium chloride in a concentration equal to that in the Warburg and Burk medium brought about considerable improvement of the special medium. The greatest improvement occurred with the addition of sodium chloride whereby the addition of merely 0.02 mg/100 ml was observed to double the amount of growth. The anion effects of sulphate, phosphate and chloride were found to vary depending upon whether the anion was added as the sodium salt or the potassium salt.

This paper reports that there was greater stimulation of growth in the special medium by chloride with the sodium salt than with the potassium salt, greater stimulation by sulphate in the presence of sodium than in the presence of potassium, and inhibition by sodium dihydrogen phosphate and no inhibition by potassium dihydrogen phosphate. Furthermore, it has been found that calcium reversed the growth inhibition produced by sodium dihydrogen

phosphate and that strontium only partially substituted for calcium.

The blue-green alga, *Anabaena cylindrica* (Allen and Arnon [1]), and bladder saltbush, *Atriplex vesicaria* (Brownell and Wood [2]), have been shown to require sodium for their growth. It may be that most or all of the blue-green algae have a nutritional requirement for sodium. Hodgson [6] investigated the possible essentiality of sodium in the nutrition of radish, barley and celery. Although he found that the yield of celery was doubled when 60 ppm of sodium was added to the culture solutions, he concluded that sodium was not essential but that it served some beneficial function. The results of Hodgson were confirmed by Woolley [21] who obtained a 12 per cent increase in the dry weight of tomato plants with additions of sodium at the rate of one millimole of NaCl per liter of solution.

There are experimental results which indicate that sodium is quite important under certain conditions of plant nutrition. This is particularly true of sugar beet [12], cotton [13], flax [10], barley [11] and red table beet [22]. Lehr [8 9] states that for sugar beets sodium may almost be deemed an indispensable nutrient element, approaching potassium in importance. A rather comprehensive review of the literature in this field is given by Lehr [8] and by Mullison and Mullison [15].

Conflicting statements occur in the literature [7 16 17] regarding a requirement for calcium by *Chlorella*. Recently Walker [18] has shown that calcium is indeed required by *Chlorella pyrenoidosa* and that the calcium requirement can be satisfied by strontium on an approximately equimolar basis.

METHODS AND MATERIALS

The Emerson strain of *Chlorella pyrenoidosa*, obtained from Dr. Jack Myers of the University of Texas, was cultured in Eyster's special autotrophic medium [4 5] which had 3200 mg $Mg(NO_3)_2.6H_2O$, 34 mg K_2SO_4 and 25 mg KH_2PO_4 per liter. The medium was supplemented with 1 ppm Fe as $FeSO_4.7H_2O$, 0.5 ppm Mn as $MnSO_4.7H_2O$, 0.02 ppm Cu as $CuSO_4.5H_2O$, 0.5 ppm B as H_3BO_3, 0.05 ppm Zn as $ZnSO_4.7H_2O$ and 0.01 ppm Mo as $(NH_4)_6Mo_7O_{24}.4H_2O$. Fisher Certified and Baker Analyzed reagents supplied the macronutrients, and Matthey Specpure compounds furnished the micronutrients.

The water was redistilled in Pyrex-glass distillation apparatus and the glassware was supercleaned by the method of Waring and Werkman [20].

The culture flasks were 300-ml Pyrex flasks with side arms, containing 100 ml of medium, plugged with cotton and covered with rubber caps. The cultures were bubbled with 5% CO_2 in air at the rate of about 35 ml/min, agitated continuously on a shaker in a room maintained at 25° C, and illuminated with approximately 1000 footcandles of daylight fluorescent light.

RESULTS

Growth of *Chlorella pyrenoidosa* in the special medium was only about 25 per cent of that in the regular Warburg and Burk medium. Additions of anions of either chloride, sulphate or phosphate in concentrations similar to the concentration of these salts in the Warburg and Burk medium greatly improved the

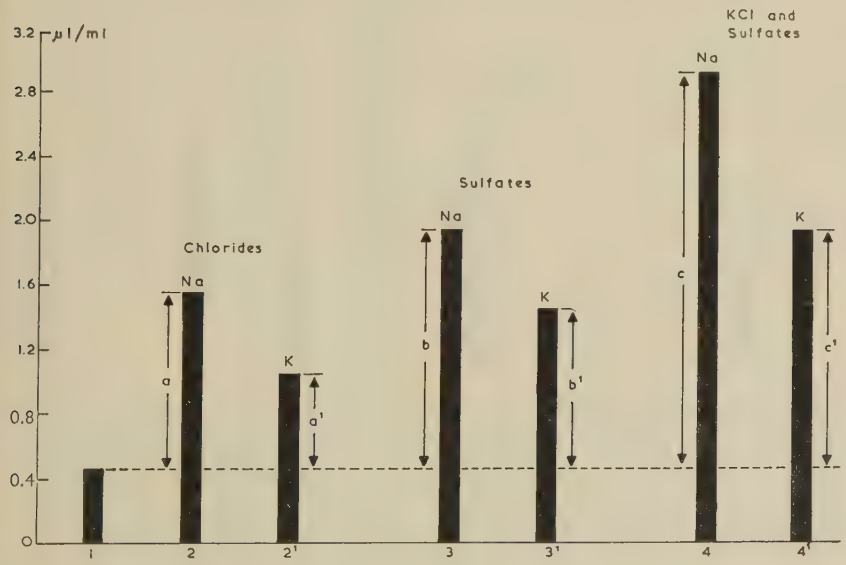

Fig. 1. Comparative growth of *Chlorella pyrenoidosa* in Eyster's special medium both with and without various anion supplements. 1. No supplement. 2. With 200 mg NaCl per 100 ml culture. 2′. With 255 mg KCl per 100 ml culture. 3. With 284 mg Na_2SO_4 per 100 ml culture. 3′. With 348.5 mg K_2SO_4 per 100 ml culture. 4. With 255 mg KCl and 284 mg Na_2SO_4 per 100 ml culture. 4′. With 255 mg KCl and 348.5 mg K_2SO_4 per 100 ml culture. Represents 3 days growth in 100 ml of medium inoculated with 0.75 µl cells which had been grown in Eyster's special medium [4] supplemented with KCl instead of NaCl and which subsequently had been washed twice in redistilled water.

special medium. Equimolar additions of sodium chloride and potassium chloride to separate cultures produced about 47 per cent more growth with the sodium salt than with the potassium salt (Fig. 1). Likewise with additions of sulphate, the sodium salt produced 34 per cent more growth than the potassium salt (Fig. 1). Combined addition of chloride as potassium chloride and of sulphate again produced more growth with Na_2SO_4 than with equimolar K_2SO_4. The growth of *Chlorella pyrenoidosa* in the special medium was greater in cultures which contained combined supplements of chloride and sulphate than in cultures with either chloride or

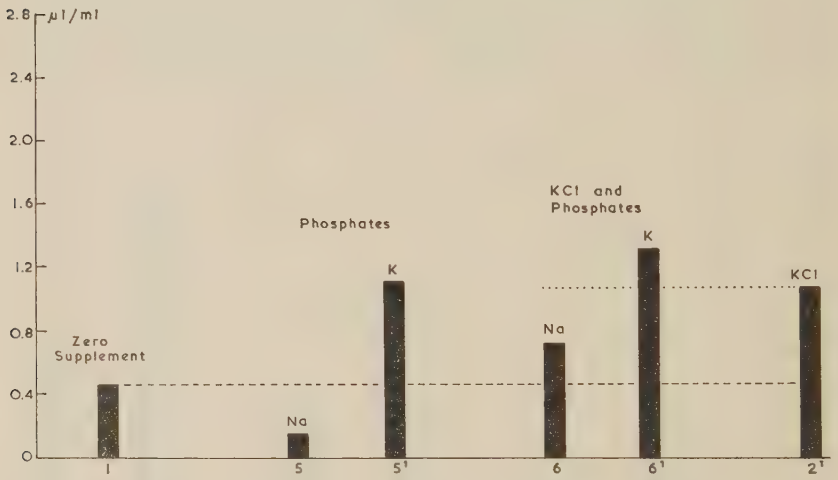

Fig. 2. Comparative growth of *Chlorella pyrenoidosa* in Eyster's special medium both with and without various anion supplements. 1. No supplement. 5. With 254 mg $NaH_2PO_4.H_2O$ per 100 ml culture. 5′. With 250 mg KH_2PO_4 per 100 ml culture. 6. With 255 mg KCl and 254 mg $NaII_2PO_4.H_2O$ per 100 ml culture. 6′. With 255 mg KCl and 250 mg KH_2PO_4 per 100 ml culture. 2′. With 255 mg KCl per 100 ml culture. Inoculum and growth period are the same as in Fig. 1.

sulphate. Generally, the former appeared to be an accumulation of the separate effects of chloride and sulphate. In Fig. 1 it can be seen that c is approximately equal to $a + b$ and that c' roughly equals $a' + b'$. Increases in growth by sodium were produced in the presence of high concentrations as well as in the presence of low concentrations of potassium. The culture medium which received the NaCl suppelment and the medium which separately received

the Na_2SO_4 supplement had 22.4 ppm K, a predetermined critical amount. The culture in which chloride and sulphate were combined had 1355.5 ppm K. Some reports in the literature [3 14 15] very definitely show increased growth by sodium in the presence of sub-critical amounts of potassium, and it was concluded that sodium can at least partially replace potassium when potassium is insufficient.

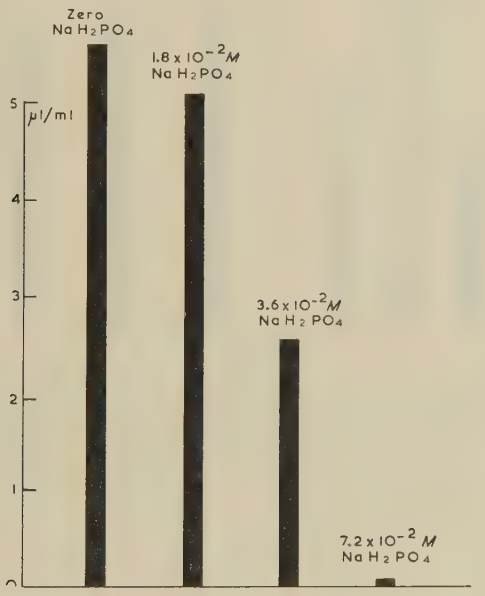

Fig. 3. Inhibition of the growth of *Chlorella pyrenoidosa* by three concentrations of NaH_2PO_4 in Eyster's special medium already supplemented with 200 mg NaCl per 100 ml culture. Represents 4 days growth in 100 ml of medium inoculated with 0.90 μl cells which had been grown in Eyster's special medium [4] supplemented with KCl instead of NaCl and which subsequently had been washed twice in redistilled water.

Phosphate also improved the special medium when this anion was added as KH_2PO_4 (Fig. 2). However, there was a definite inhibition of growth in the presence of equimolar NaH_2PO_4 (Fig. 2), which still occurred in the combined presence of chloride and phosphate.

Sodium phosphate inhibition has been shown to increase with higher concentrations of $NaH_2PO_4.H_2O$ (Fig. 3). Supplements of

NaH_2PO_4 were superimposed on the special medium which already contained the critical concentration of phosphate ($1.8 \times 10^{-4} M$) added as KH_2PO_4 and furthermore had been improved by the addition of 200 mg NaCl/100 ml.

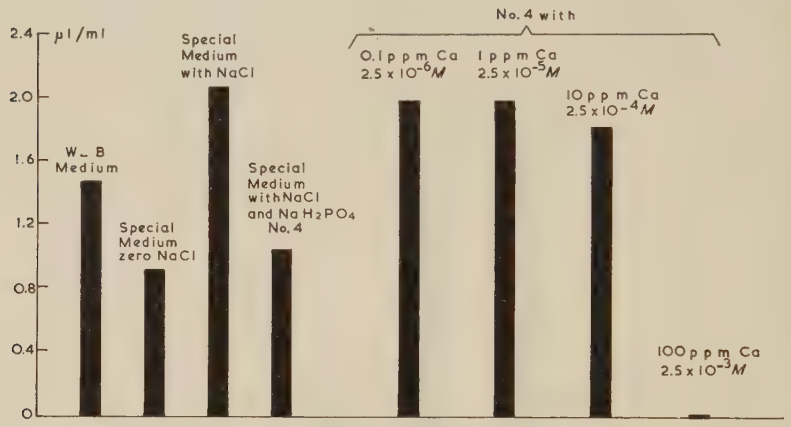

Fig. 4. Comparative growth of *Chlorella pyrenoidosa* in Warburg and Burk medium, in Eyster's special medium, in Eyster's special medium supplemented with 200 mg NaCl per 100 ml culture, and in the latter medium further supplemented with 254 mg $NaH_2PO_4.H_2O$ per 100 ml culture (designated as No. 4). The effects of 0.1 ppm, 1 ppm, 10 ppm and 100 ppm calcium when added to No. 4 are shown. Inoculum and growth period are the same as in Fig. 3.

In a preliminary experiment (Fig. 4) it was found that calcium could almost completely eliminate the inhibition produced by NaH_2PO_4. Calcium concentrations of 0.1 ppm, 1 ppm, and 10 ppm proved to be about equally effective in overcoming the inhibition. However, the culture which had been given 100 ppm calcium produced very little growth. Furthermore, Figure 4 compares the growth of *Chlorella* in Warburg and Burk medium with that in the special medium prepared either with or without the usual supplement of NaCl.

A more thorough study of the effect of calcium in overcoming NaH_2PO_4 inhibition and of the question whether strontium can replace calcium in this effect is given in Figure 5. The minimum concentration of calcium which produced maximum reversal of the NaH_2PO_4 inhibition was shown to be $2.5 \times 10^{-5} M$ (1 ppm Ca).

The critical concentration range where more and more calcium produced more and more reversal lay between 2.5×10^{-7} *M* and 2.5×10^{-5} *M*. Calcium was not effective in this reversal at concentrations of 2.5×10^{-7} *M* and below. Strontium could only

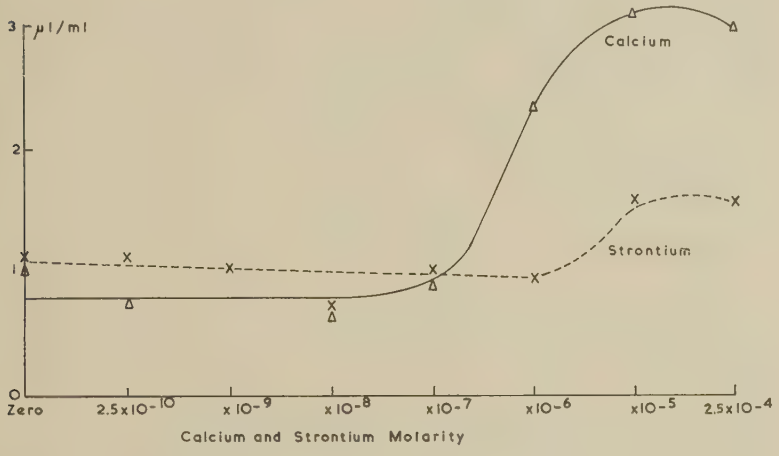

Fig. 5. The effect of various concentrations of calcium in overcoming the inhibition of the growth of *Chlorella pyrenoidosa* produced by 3.6×10^{-2}M NaH_2PO_4 and a study of whether strontium can replace calcium in this effect. Inoculum and growth period are the same as in Fig. 3.

partially replace calcium. This result is contrary to the findings of Walker [18] who reported that strontium was more effective than calcium in promoting the growth of *Chlorella* in the presence of ethylene diamine tetra acetic acid. The comparative effects of calcium and strontium are given in Figure 5.

SUMMARY

The growth of *Chlorella pyrenoidosa* in a special medium based on the critical concentrations of nutrients for autotrophic growth has been shown to be stimulated more by chloride with the sodium salt than with the potassium salt, more by sulphate in the presence of sodium than in the presence of potassium, and to be inhibited by sodium dihydrogen phosphate and not by potassium dihydrogen phosphate. Furthermore, it has been found that calcium reversed the growth inhibition produced by sodium dihydrogen phosphate, and that strontium only partially substituted for calcium.

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SULPHUR OXIDATION IN TIDAL MANGROVE SOILS OF SIERRA LEONE

by M. G. R. HART

West African Rice Research Station, Rokupr, Sierra Leone

INTRODUCTION

The development of mangrove soils for rice cultivation in Sierra Leone has been proceeding for about seventy years, and much of the rice now produced in the territory is grown on these soils. The system used is one of free tidal irrigation: during both wet and dry seasons tidal water is allowed to flow freely on and off the land. In the dry season, when the water in the rivers becomes saline, high salt concentrations develop in the soils but these are quickly reduced at the onset of the following rainy season, before rice is transplanted. The soils contain large amounts of sulphur in various forms, and although fluctuations occur in the pH value of the surface soil (Tomlinson [20]), such fluctuations do not interfere with the growing of a rice crop during the rainy season.

The tidal irrigation system is limited to those areas lying on main rivers or creeks which carry sufficient fresh water during the rains to push back the sea water and leach the accumulated salt from the soil. Future expansion of the rice-producing capacity of the territory will depend to some extent on the development of permanently saline *Rhizophora* mangrove swamps in coastal areas. Large tracts of this type of land are at present uncultivable owing to their high salinity and to the extremely acid conditions which develop in them if they are empoldered to keep out salt water (Dent [4], Tomlinson [19]). Owing to the absence of any appreciable source of indigenous liming material in Sierra Leone, the use of heavy dressings of lime to neutralise the acid formed in empoldered soils would be very expensive. On the other hand, experience has

shown that even when such soils are subjected to heavy drainage, recovery is slow: at Wellington, in the Sierra Leone Colony, a ten-year period elapsed between the completion of the empolder and the production of a satisfactory rice crop over the whole of the empoldered area.

The formation of acid by sulphur oxidation has been reported previously in a variety of soils (Jensen, Drewes, Harmsen cf. Starkey [15]; Wiklander, Hallgren, Brink and Jonnson [23]; Quispel, Harmsen and Otzen [12]; Chenery [1]; Harmsen, Quispel and Otzen [7] and in drainage from disused bituminous coal mines (Leathen, Braley and McIntyre [9]; Temple and Delchamps [17]; Temple and Koehler [18]). The present investigation was undertaken in order to obtain information on the way in which acid conditions develop in tidal mangrove soils, with the hope that such information might lead to the formulation of suitable means of economic reclamation.

METHODS

The *soil* used in the experiments was collected from a silted drain on the Rokupr Experimental Farm, which is under tidal conditions throughout the year. Collections were always made from a depth of about one foot owing to seasonal variations in the sulphur content of the surface mud, which is well leached and may be altered in composition by the deposition of river-borne silt.

In experiments on the effect of drying on soil acidity and other factors, the soil was brought into the laboratory and allowed to dry at room temperature in the air. It was thoroughly mixed every day throughout the experimental period.

Soil *pH value* was determined in 1 : 1 water suspension using a pH meter and glass electrode.

Titrateable acid was determined by titrating 5 g soil in 15 ml distilled water with $N/10$ NaOH, the alkali being added in 1-ml aliquots and the mixture stirred for 5 min before the pH value was determined.

Sulphate was determined by the method of Chesnin and Yien [3], possible interference from colloidal organic matter being eliminated by co-precipitation with ferric hydroxide as recommended by Hesse [8]. Soluble sulphate was extracted with Morgan's reagent (sodium acetate – acetic acid mixture buffered to pH 4.5) and total sulphate with conc. HCl, which dissolves the basic ferric sulphates which are common constituents of dried mangrove soil.

Forms of sulphur other than sulphate were determined by the methods of Smittenburg *et al.* [13].

Iron (Fe^{++} and Fe^{+++}) was determined using α–α' di-pyridyl reagent.

Viable counts of sulphur-oxidising organisms were made from serial tenfold dilutions in physiological saline solution. Eight bacteriological test tubes containing sulphur medium (Starkey [14]) were inoculated at each dilution level. The tubes were incubated on their sides for three weeks at room temperature (average 27° C), after which the media were tested with methyl-orange solution. The most probable number of organisms present in the original soil sample was calculated from the number of tubes in which acid had been formed, using the tables of Fisher and Yates [5].

EXPERIMENTAL RESULTS AND DISCUSSION

(a) *Preliminary analyses*

Samples of the soil were analysed for oxidisable sulphur before and after extraction with acetone. The results are shown in Table 1 (samples 1–6). Considerable variability existed between samples, especially in the proportion of extractable sulphur present. This

TABLE 1

Results of preliminary analyses of mangrove soils and detritis

Sample	Total oxidisable S mg/g		Residual S mg atoms/g	Total Fe mg atoms /g	HCl-soluble Fe mg atoms/g		Residual Fe mg atoms/g		S/Fe ratio	
	Initially	After acetone extraction			a *	b *	a	b	a	b
l										
1	17.3	7.3								
2	11.9	5.6								
3	20.7	7.3								
4	16.8	9.1								
5	17.6	16.8								
6	16.2	8.4								
Iean 1–6	16.8	9.1								
vitis										
7	76.0	53.0								
8	77.3									
9	160.0	130.4								
10	43.5	39.6	1.24	0.65	0.13	0.17	0.52	0.48	2.4	2.6
11	25.4	20.0	0.62	0.58	0.28	0.42	0.30	0.16	2.1	3.9
12	45.1	25.9	0.81	0.86	0.36		0.50		1.6	
13	55.4	40.7	1.27	0.99	0.21	0.34	0.78	0.65	1.6	2.0
14	40.5	34.5	1.08	0.61	0.16	0.24	0.45	0.37	2.4	2.9
15		39.0	1.22	0.81	0.28	0.42	0.53	0.39	2.3	3.1

a = iron extraction by boiling for 10 min in HCl (1 : 1).

b = iron extraction by heating on a steam bath with HCl (1: 1) for 2 hours. Mixture left overnight.

was surprising, as the sampling was carried out by vacuum drying 50 g soil and grinding it in a mortar before a small sub-sample was taken for analysis. Agreement between such sub-samples was normally of the order of 5 per cent. It was concluded that the sulphur was unevenly distributed throughout the soil, and an attempt was made to discover the source of the variability by searching the soil for lumps or aggregates. The analysis of flakes of blackened decayed wood found in the soil revealed that these were heavily impregnated with oxidisable sulphur (samples 7–15), and it was thus concluded that they were the source of the variability The wood was probably the remains of the mangroves which had been cleared from the land before rice was cultivated, but it was shown that even old palm-nut shells recovered from the soil had served as sites for the accumulation of sulphur. The result is of interest as Partington [11] has described the occurrence of pyrites in coal in the form of "roots, wood etc.".

Extraction of the material with acetone suggested that a large proportion of the sulphur present was in the form of polysulphide. An attempt was made to confirm this by determining the S/Fe (mg atoms) ratios in the samples after removal of the acetone-soluble sulphur and iron soluble in 1 : 1 HCl. Two separate extractions with 1 : 1 HCl were carried out: (a) by boiling for 10 minutes and (b) by heating for two hours on a steam bath and allowing the mixture to stand overnight. S/Fe ratios determined using method (a) were scattered about 2.0, the expected value if all the sulphur present after acetone extraction was as FeS_2. However, the results from method (b) gave somewhat higher values, and until more detailed studies can be carried out it will not be possible to determine with certainty the size of the polysulphide fraction, although the results give an indication that polysulphide was present in some quantity.

It is not known at present how polysulphide formation occurred, but it seems likely that preliminary accumulation took place as ferrous monosulphide, while the organic matter was fresh and the conditions reducing. Subsequent formation of polysulphide sulphur may have taken place at a later stage, possibly when the conditions were less reducing. It is of interest to note that accumulation of monosulphide in association with mangrove remains has been repeatedly observed in freshly cleared swamps.

The mean content of total oxidisable sulphur of samples 1–6 is in good agreement with the total-sulphate contents found in subsequent experiments. The mean of the residual sulphur contents after acetone extraction may be high, either on account of the unusually high value in sample 5 (possibly the result of sampling error), or because of the possibility that the whole of the extractable-sulphur content was not extracted. (Periods of extraction of up to 24 hours at room temperature were employed and the acetone was changed three times during the extraction).

Analyses for monosulphide sulphur showed that this was not normally present in excess of 500 ppm oven-dry soil. Sulphate sulphur was not found in significant quantity in freshly collected soil.

(b) *Rate of drying of the soil and its fall in pH value*

The rate at which the soil dried, and its corresponding fall in pH value are shown in the graph plotted in Fig. 1. The increase in the

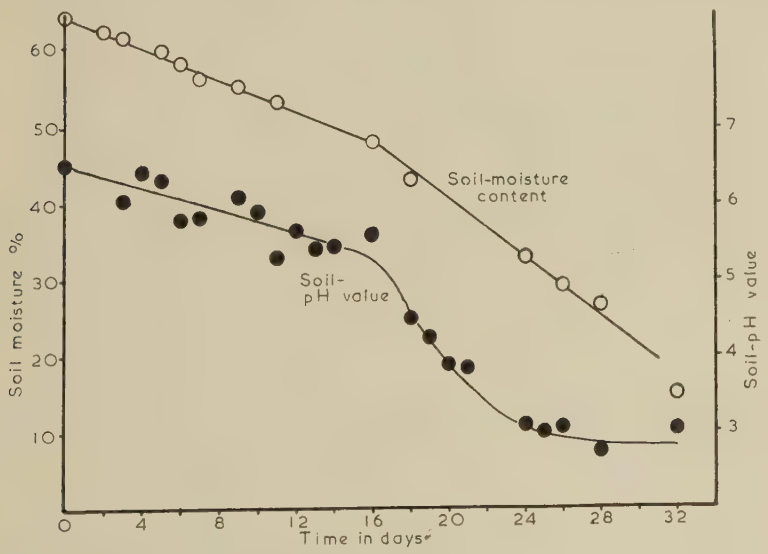

Fig. 1. Graph showing relationships between rate of fall of soil-moisture content and soil pH value.

rate of soil-moisture loss which occurred after eighteen days was due to the separation of the soil mass into aggregates, thus increasing the surface area available for evaporation. At this point

the soil-pH value began to fall steeply. The length of the initial lag period varied from experiment to experiment, according to the humidity of the atmosphere and the amount of soil used, but the change in the rate of soil-moisture loss always coincided with the beginning of the rapid fall in soil-pH value. It seems that this fall began immediately after the establishment of aerobic conditions in the soil, and that such factors as bacterial population and oxidisable sulphur did not limit the initiation of acid formation. The results obtained using shaken soil–water suspensions, in which no lag occurred, confirmed this.

(c) *The effect of sodium azide on the fall in soil-pH value*

Wiklander *et al.* [23] in their study of Swedish "gyttja" soils, concluded that although sulphate formation in these soils was carried out by both chemical and biological means, biological oxidation was likely to be dominant under field conditions. In their experiments, Wiklander *et al.* showed that favourable soil-moisture contents much enhanced the rate of sulphate formation by stimulating biological activity. The conditions under which the present experiments were performed would be expected to provide ideal moisture conditions for bacterial activity at some stage in the drying process, and would thus be expected to be favourable to the development of a large and active bacterial population. The way in which the experiments have been carried out is likely to approximate fairly closely to field conditions in Sierra Leone, as soil acidity develops in empoldering schemes during the months of January and February, when the soil is drying out after the rainy season.

Sodium azide has been used successfully by Gleen and Quastel[6] to inhibit thiosulphate-oxidising bacteria and by Temple and Colmer [16] to prevent oxidation of iron by the bacterium *Thiobacillus ferro-oxidans*. In the present investigation, 0.2% sodium azide was added to separate 1-kg samples of mangrove mud 0, 2, 4, 6 and 8 days after collection and a control was left to dry without the addition of azide. The results of pH determinations on the different treatments are plotted in the graph shown in Fig. 2. The sample with azide added after 2 days behaved identically with that to which azide was added at the beginning, and the results are therefore not included. Analysis of azide-treated soil for sulphate showed that sulphate formation was also delayed by treatment.

The samples used in this experiment were much smaller than those which have been normally used, and for this reason the rapid fall in the soil-pH value began earlier than usual. The additions of azide were effective in preventing the pH value from falling until a much later stage, and reduced the intensity of acid formation by about one pH unit. Remoistening azide-treated soils followed by further air drying had no effect on their pH values. The results indicate clearly that under the experimental conditions, acid formation was entirely dependent upon bacterial activity, and that purely chemical oxidation may be effective only at lower soil moisture contents. It is to be expected from these results that

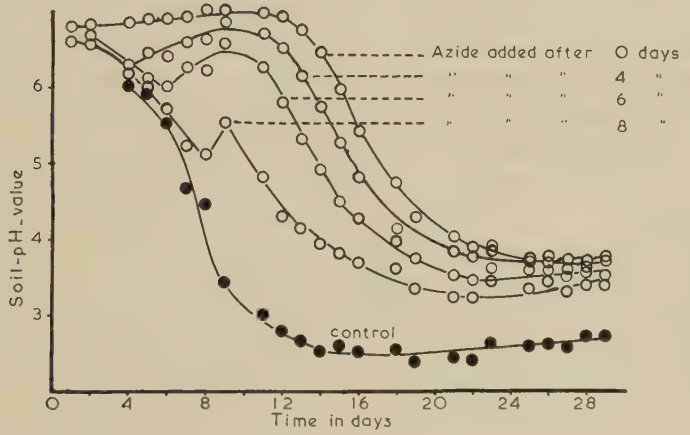

Fig. 2. Graph showing the effect of additions of sodium azide on the fall in soil-pH value.

bacteria will be of major importance in acid formation in the field, as their optimum soil-moisture content is likely to be reached before that which favours chemical oxidation.

(d) *Sulphur-oxidising bacteria and acid formation.*

The importance of *Thiobacillus thio-oxidans* in the production of high acidities in soils and waters has been widely reported (Starkey [15]). An attempt was therefore made to discover whether there was any correlation between the number of sulphur-oxidising bacteria present in the soil and the rate of fall of the soil-pH value. That such a correlation did exist is evident from the results, which

are plotted in the graph shown in Fig. 3. Increase in the number of organisms present can also be seen to be related to the increase which occurred in the soluble-sulphate content of the soil. In this experiment, sulphate was not determined by extraction with conc. HCl and the figures given, which represent the sulphate extracted with Morgan's reagent, probably account for only part of the total sulphate content.

The highest number of organisms recorded in this experiment was lower by about a factor of ten (*i.e.* just significantly lower) than the number estimated to be present in a column of soil through

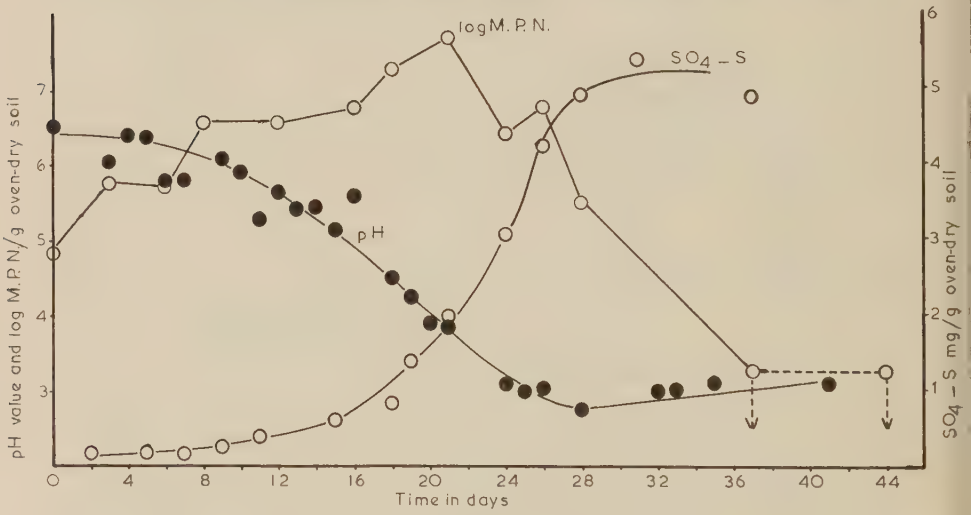

Fig. 3. Graph showing the relationships between the number of sulphur-oxidising organisms present, the rate of soluble-sulphate formation, and the fall in soil-pH value. (M.P.N. = most probable number).

which thiosulphate was being perfused, when the rate of oxidation in the column had reached its maximum. The soil was thus at this stage approaching "bacterium saturation" (Lees and Quastel [10]), and conditions were almost ideal for bacterial sulphur-oxidation. The results confirm that the formation of acid in the soils is a direct consequence, at least in its final stages, of the activities of the sulphur-oxidising bacteria. Bacteria cultured from dilution tubes grew best in sulphur medium below pH 5 and produced final pH values of about 0.6. Their characters corresponded with those of *Thiobacillus thio-oxidans*.

The rapid decline which occurred in the number of organisms present after the maximum had been reached could not be attributed to exhaustion of the supply of oxidisable sulphur, as the preliminary analyses had shown that this was normally present in sufficient quantity to account for more soluble sulphate than was actually produced. It also seemed unlikely that moisture was the limiting factor, at least in the early stages of the decline, and this was borne out by remoistening the soil immediately after the minimum pH value had been reached. No increase in soluble-sulphate content or decrease in pH value occurred in the remoistened soil. It thus seemed likely that the low soil-pH value was responsible for the decline, though any effect of pH would have to be an indirect one as the bacteria produced much lower pH values in culture media than in the soil.

The fact that the soil invariably attains a pH value of between 3.0 and 2.4 after drying suggests that iron may be involved. Wiklander *et al.* [23] obtained a marked stimulation of sulphate formation in gyttja soils by heavy dressings of lime. The final pH values in the soils were normally between 3.0 and 2.0, and it is possible that inhibition of the sulphur-oxidising bacteria occurred as a result of the increased availability of ferric iron between these values. Liming would delay the fall in pH value and increase the length of time available for bacterial sulphur oxidation before the limiting pH value was reached. This explanation is supported by the fact that, in Wiklander's experiments, the effect was more marked the higher the lime rate.

Chenery [1], working on papyrus-peat soils in Uganda, demonstrated that when the soils were dried their surface layers did not attain such low pH values as their deeper layers. The surface soil contained much larger concentrations of total (free) iron than the sub-soil, and the effect was attributed to buffering by the iron. However, Chenery's results would not exclude the possibility that iron inhibition of the sulphur-oxidising bacteria was involved. The effect noted in mangrove soils is unlikely to be due to chemical buffering, which would not be expected to cause a decline in the rate of sulphate formation or in the numbers of sulphur-oxidising bacteria present.

(e) *Changes in extractable iron during drying*

In his study of the toxicity of acid mangrove soils to rice plants, Tomlinson [21] showed that large amounts of iron and aluminium could be extracted from these soils when their pH values were less than 5.0. These results were correlated with the restriction of rice growth which occurred below this pH value. It was suggested that one or both of these ions was responsible for the toxicity of the soils.

In order to obtain an indication of the concentrations of the ions actually available to the plants, Tomlinson used a normal solution of ammonium acetate with the pH value adjusted to within 0.2 units of that of the soil to be extracted. This technique had been used successfully by Chenery [2] in his study of the availability of aluminium in tea soils, and it seems likely that Tomlinson's results gave a good indication of the pH value at which aluminium toxicity would be expected to occur in mangrove soils. However, the results obtained for iron may not give a true picture of its availability in the soil on account of its high solubility in the extract at relatively high pH values. Large concentrations of ferric iron can be extracted from mangrove soil using an acid (pH 2.8) ammonium-acetate extractant regardless of whether the soil itself is acid or not. It may thus be argued that results obtained using extracts with their pH values adjusted to be near that of the soil are dependent on the acidity of the extract rather than that of the soil.

It was decided that for the purposes of the present investigation extraction of the soil with 0.2 *M* KCl might give a better indication of the stage during the drying of the soil at which iron is likely to become available in toxic concentrations, both to rice and to sulphur-oxidising bacteria. The KCl extractant would be expected to attain the pH value of the soil and should therefore provide information on the solubility of iron in the soil solution, although as Chenery pointed out, extraction with a neutral salt solution tends to lower the pH value of the mixture below that of the soil solution, and might thus increase the apparent availability of the extracted ion.

The results of analyses of KCl extracts of mangrove soils during drying are shown in the graph plotted in Fig. 4. Concentrations of ferrous and ferric iron only are shown. Aluminium was also determined; its concentration rose steeply after 21 days as the soil-pH

value fell below 4. This would be expected if the extractability were dependent solely on the solubility of aluminium hydroxide. Results for aluminium are not included in detail as it does not appear to be involved in the limitation of the activity of the sulphur-oxidising bacteria.

A steady increase in the amount of ferric iron extractable from

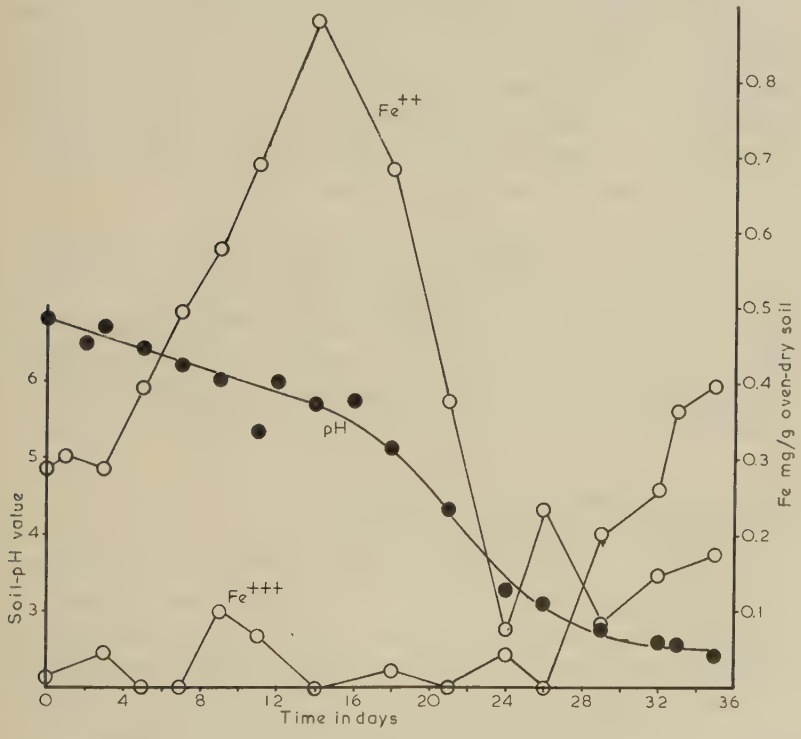

Fig. 4. Graph showing the amounts of ferrous and ferric iron extractable by 0.2 *M* KCl at different soil-pH values.

the soil occurred after the soil-pH value had fallen below 3.0. Above this pH value ferric iron could not be extracted using KCl and it was concluded that the extractability of ferric iron was dependent on the expected solution of ferric hydroxide at about pH 3. The pH value at which ferric iron comes into solution thus corresponds with that at which the decline in the activity of the sulphur-oxidising bacteria occurs. It also seems likely from these results

that aluminium would be more important in the toxicity of mangrove soils than ferric iron.

The increase in the amount of extractable ferrous iron which occurred during the lag period was at first thought to be due to an increase in the solution of soil organic matter able to chelate the ferrous iron and protect it from oxidation during extraction. However, estimations of the organic-carbon content of the extracts showed that although the amount extracted did increase concurrently with the increase in ferrous iron, the amount of carbon present was insufficient to account for the effect, the ratio of increase in C to increase in Fe being just greater than 2. Furthermore, extraction of the same soil with carbonate–bicarbonate mixture (pH 9.1) yielded only traces of iron after destruction of organic matter by tri-acid digestion.

An alternative explanation for the increase in the amount of ferrous iron extracted during the lag period might be the release of soluble ferrous iron by hydrolysis from insoluble ferrous monosulphide. This would be expected to occur during this period and the actual quantity of ferrous iron released is compatible with the normal initial content of monosulphide sulphur in the soil.

(f) *The effect of $CaCO_3$ on sulphur oxidation*

Wiklander *et al.* [23] have reported that early investigations gave contradictory results on the effects of lime on sulphur oxidation. In Wiklander's own experiments, a straightforward stimulation of sulphate formation was obtained, and, as has already been mentioned, the results of the present investigation suggest that such a stimulation could be due to the effect of the lime in delaying the development of a soil pH inhibitory to the activity of the sulphur-oxidising bacteria. An experiment was therefore carried out to determine whether a stimulation of sulphate-formation in mangrove soils could be obtained by treating them with liming material after the decline in bacterial activity and sulphate-formation had begun.

Fresh soil was allowed to dry slowly in the air as in previous experiments. After 35 days, when the moisture content had fallen to about 30%, the soil was divided into three equal parts. One part was left untreated and the other two were remoistened to 47% moisture content with distilled water. One of the moistened samples was treated with $CaCO_3$ at the rate of 10 mg/g

air dry soil. All three samples were then left in the air until the moisture contents of the moistened samples had fallen to about 40% (the water content at which the maximum rate of fall of soil pH value normally occurred). The containers were then covered with polythene sheeting to minimise further water loss. The soils were mixed daily as before.

TABLE 2

The effect of remoistening and treatment with $CaCO_3$ on sulphur oxidation in dried mangrove mud *

Time in days	pH	% H_2O	SO_4–S soluble	SO_4–S total	log M.P.N. **
Soil left dry					
35	2.41	31.6	7.3		6.17
42	2.47	27.4	8.4		
45	2.36	25.1	7.7		
49	2.61	25.3	7.7		
52	2.38	25.0	7.3		
59	2.41	24.8	7.3	15.6	
Soil remoistened					
35		47.5			
42	2.72	41.2	7.1		7.00
45	2.42	38.5	7.2		3.45
49	2.54	39.3	7.0		4.22
52	2.53	39.0	7.0		3.70
59	2.29	38.8	7.4	16.0	2.98
Soil remoistened + $CaCO_3$					
35		47.2			
42	3.38	40.2	8.4		6.99
45	3.09	38.8	8.8		6.57
49	3.02	38.5	9.9		6.70
52	2.95	38.1	9.1		6.10
59	2.75	38.0	8.6	16.8	5.84

* S expressed as mg/g (oven-dry) soil.

** M.P.N. = most probable number of organisms per g (oven-dry) soil.

The results of determinations carried out during the experiment are shown in Table 2. They may be summarised as follows:

1. Liming and remoistening the soil increased the soluble-sulphate content and maintained the number of sulphur-oxidising organisms at a high level.

2. There was an increase in the total-sulphate content of the limed soil over that of the soil left dry, and to a lesser extent over that of the remoistened unlimed soil. The increase was not sufficient to account for the increase in soluble sulphate and may not be significant.

It is suggested that the effect of lime was similar to that noted by Wiklander, and the maintained activity of the sulphur-oxidising organisms adds weight to the ideas already expressed on the mechanism of this effect. It was shown in a subsidiary experiment that the amount of iron extractable by 0.2 *M* KCl from an acid soil was reduced from 600 ppm to 0 ppm by liming with the same quantity of $CaCO_3$ as was used in the above experiment.

These results differ from those of Wiklander *et al.* in the magnitude of the effect. Whereas an increase of over 200 per cent was obtained in the sulphate content of gyttja soil from Ersnas by liming at the rate of about 8 mg/g dry soil, the stimulation in the mangrove soil amounted to less than 30 per cent. A possible explanation for this might be that the two soils differ in the type of sulphur compounds which they contain. The gyttja soil contained 10 mg/g dry matter as acid-soluble sulphide out of a total sulphur content of 13 mg/g (Wiklander *et al.* [22]). Both Verhoop (cf Harmsen *et al.* [7]) and Wiklander *et al.* [23] have envisaged the decomposition of ferrous sulphide as occurring in two phases, a preliminary rapid oxidation of the iron to hydroxide and a subsequent bacterial oxidation of the elemental sulphur released to sulphuric acid. The experiments of Wiklander *et al.* [23] showed that when gyttja soils were kept under dryish conditions which would be unfavourable for the development of a large population of sulphur oxidisers, sulphate formation was slow. This would be expected from the above scheme for the oxidation of iron monosulphide. The rate of sulphate formation and the amount of sulphate eventually formed in the gyttja soil would be expected to be largely dependent upon bacterial action, and a large stimulation would be expected from applications of liming material. On the other hand, the analyses already described have shown that only a proportion of the total sulphur content of mangrove soils is present in a form susceptible to immediate bacterial decomposition and acid formation, and it may be that the oxidation of only this fraction is stimulated by the addition of calcium carbonate. From the results of Quispel *et al.* [12] and Temple and Delchamps [17] it would be expected that the pyrites fraction would be oxidised rapidly only at low pH values. This fraction may have already been oxidised after the first 35 days, with the formation of insoluble sulphate. The effect of the liming material added after 35 days

would thus be limited to the non-polysulphide fraction of the soil sulphur content. A further experiment was therefore carried out to discover whether the oxidation of the pyrites fraction could be limited if $CaCO_3$ was added immediately after the soil was collected. Six samples of freshly collected soil, each of 500 g, were treated with $CaCO_3$ at the rate of 0, 2, 4, 6, 8, and 10 mg/g fresh soil and allowed to dry. The results of analyses carried out at intervals on samples of the various treatments are given in Table 3. Titrateable acid was determined at the end of the experiment by titrating a sample of soil with standard alkali as already described, and allowing for the $CaCO_3$ added.

TABLE 3

The effect of $CaCO_3$ on sulphur oxidation during drying of mangrove mud. Initial soil pH = 6.79. Initial soil-moisture content = 60.5%. S expressed as mg/g (oven dry) soil

$CaCO_3$ added (mg/g wet soil)	pH	% H_2O	Oxidisable S	SO_4-S soluble	SO_4-S total	Titrateable acid as H_2SO_4 in mg S per g oven-dry soil
After 32 days						
0	2.49	38.9	2.8	7.1		
2	2.46	39.1		8.4		
4	2.88	36.8		8.1		
6	5.71	38.1		3.4		
8	7.38	39.0		3.0		
10	7.55	36.8	12.2	2.6		
After 42 days						
0	2.60	30.5	1.1	9.8		
2	2.54	29.6	4.0	11.1		
4	2.67	28.6	0.9	10.9		
6	3.38	30.5	5.3	8.1		
8	6.07	33.1	7.3	5.0		
10	6.52	24.2	9.2	4.0		
After 55 days						
0	2.37	13.6		7.8	15.1	7.8
2	2.53	10.7		9.5	15.1	8.7
4	2.54	9.1		10.1	14.7	8.8
6	3.12	9.7		9.0	10.9	8.0
8	5.42	10.6		0.0	5.9	5.3
10	6.62	8.0		0.0	5.1	5.4

There were three effects of the liming material on sulphate and acid formation. They may be summarised as follows:

1. A stimulation of soluble-sulphate formation. This occurred

in the 2-, 4-, and possibly 6-mg treatments, and was reflected in the amount of titrateable acid formed, though not in the final total-sulphate content.

2. An inhibition of soluble-sulphate formation. This was noted in the 8- and 10-mg treatments where both the soluble-sulphate content and the final total-sulphate content were lower than in the control. This effect also occurred in the early stages in the 6-mg treatment, but the final soluble-sulphate content was of the same order as that of 2- and 4-mg treatments. The titrateable acid in the two soils with 8 and 10 mg $CaCO_3$ was lower than that in the remainder.

3. An inhibition of final total-sulphate content. This occurred in the last three treatments, and in the 8- and 10-mg treatments was probably a combination of two effects. The effects may be explained in the following way.

The stimulation of soluble-sulphate formation which occurred could be attributed to the stimulatory effect of liming material on the activity of the sulphur-oxidising bacteria, which has already been demonstrated.

The inhibition of soluble sulphate formation in the 8- and 10-mg treatments may also be the result of the effect of the $CaCO_3$ on the activity of the bacteria, but this time in the opposite direction. In sulphur medium, above pH 6, the organisms were unable to grow. The effect of lime in this instance might be in maintaining the soil pH value at a high level unsuitable for rapid development of *Thiobacillus thio-oxidans*. A general slowing down of the rate of acid formation would thus occur until the pH value fell sufficiently to allow sulphur oxidation by *Th. thio-oxidans*. This probably occurred in the 6-mg treatment. In the 8- and 10-mg treatments the pH value remained high throughout the experiment, or at least until the soil-moisture content was too low to allow a high rate of bacterial acid formation. The fall of the soluble-sulphate content to zero in the last two treatments is probably due to the formation of insoluble sulphate, a reaction possibly favoured by the high pH value.

The inhibition of the final total-sulphate content which occurred in the 6-, 8-, and 10-mg treatments may be partly due to the effect described above. However, a third effect, that of inhibition of

pyrites oxidation may be involved. After 32 days, the total-sulphur content of the control, as given by the sum of the soluble-sulphate and oxidisable-sulphur contents, was much less than that of the 10-mg treatment as given by the same means. This indicates that the balance of the soil sulphur had been oxidised and precipitated out as insoluble sulphate. The results obtained after 42 days are similar, except that the 2-mg treatment spoils the pattern – this might be due to unfortunate sampling. There is an indication then, that a certain fraction of the soil sulphur is oxidised to insoluble sulphate only under acid conditions. This fraction would be very roughly about 6 mg/g oven dry soil, and this figure would be in good agreement with the content of polysulphide found in the majority of the soil samples examined in the preliminary analyses. The treatment which received 6 mg $CaCO_3$/g soil remained above pH 3 throughout the experiment, and although the soluble-sulphate content was comparable with that of the 2- and 4-mg treatments, the total-sulphate content was lower by about 4 mg/g. This could be due to the fact that although the pH value of this soil fell sufficiently to allow normal bacterial formation of soluble (acid) sulphate from the labile sulphur fraction (presumably elemental sulphur), it did not reach the low value necessary for the decomposition of the majority of the pyrites present, and this fraction thus remained un-oxidised. These suggestions would fit in with the results of Quispel *et al.* [12] mentioned previously. The treatments showing this effect did not show a corresponding reduction in the amount of titrateable acid formed, and it might be concluded that the pyrites fraction of the soil sulphur was not significantly involved in acid formation in the mangrove soils under investigation, although it could obviously be so involved from the results of Quispel *et al.* and Temple and Delchamps [17].

GENERAL DISCUSSION

Under the experimental conditions, bacterial acid formation was of major if not sole importance. It appears that purely chemical acid formation may be important only at lower soil-moisture contents. During the dry season in Sierra Leone, conditions in the field would favour bacterial sulphur oxidation as the moisture content of the surface soil falls gradually from field capacity to a

very low value. Dilution counts made on samples of soil from a plot of land under rice cultivation have shown that a large increase in the number of sulphur-oxidising organisms present in the soil occurs during the early months of the dry season when the soil pH value is falling. This may be partly responsible for the pH fluctuations which were noted by Tomlinson [20].

The development of a large and active population of *Thiobacillus thio-oxidans* appears to be controlled only by the soil-moisture content. Adequate supplies of readily oxidisable sulphur (*i.e.* elemental or monosulphidic) are available, and the soil, even in its waterlogged condition, contains a large basal population of organisms which may be enriched as soon as favourable conditions of aeration develop. Sulphur-oxidation in the soil seems to proceed in much the same way as in a soil percolation apparatus, though the amount of acid eventually produced depends on the buffering capacity of the soil between its fresh pH value and the pH value at which the activity of the sulphur-oxidising bacteria is restricted by the increased availability of ferric iron.

The results obtained from experiments with liming material may go some way towards explaining the contradictory results of early investigators which have been reported by Wiklander *et al.* [23]. Liming material appears to have two main effects, an effect on the sulphur-oxidising bacteria which may stimulate or inhibit sulphur oxidation according to whether the soil-pH value is moved into or out of their range of activity, and an effect on the decomposition of pyrites, which appears to be stable to oxidation above pH 3. From the practical point of view, Wiklander *et al.* [23] suggested that the stimulatory effect of lime on sulphur oxidation might help in the amelioration of gyttja soils by ensuring that all the soil-sulphur reserves were oxidised to sulphate in a short time, so that the soil could be rapidly reclaimed by leaching. This approach would be too expensive in Sierra Leone, where the object should be to limit sulphur oxidation as much as possible so that gradual elimination of the sulphur compounds can be effected over a longer period, without the development of high soil acidity. The results of the present investigation indicate that, in order to prevent the development of acid conditions in the soil used, a dressing of liming material almost as large as that required for complete neutralisation would be needed. In such a soil, prevention would thus be just as

expensive as cure, and probably less of a certainty. However, it has been found that some mangrove swamps in Sierra Leone can be empoldered without fear of the development of high acidities. Such swamps are usually cleared from a different type of mangrove, mainly *Avicennia nitida*, which often grows on slightly higher land where the soil may be sandy and is sometimes only flooded at high spring tides. It may be that the greater permeability of such soils is effective in controlling or eliminating the formation and retention of monosulphide and elemental sulphur, at least in the surface layers of the soil. Accumulation of pyrites is likely to occur in these soils as Harmsen *et al.* [7] have reasoned that pyrites formation must take place under aerobic conditions, and have demonstrated that this substance is actually formed under aerobic conditions in freshly suspended marine detritis. A possible solution to the problem of the development of saline *Rhizophora* swamps in Sierra Leone might lie in the preliminary establishment of soil conditions (possibly by improved drainage or selection of suitable areas) which would favour pyrites formation before empoldering. The oxidation of pyrites could then possibly be controlled by an economic dressing of lime large enough to neutralise the acid formed from residual elemental and monosulphide sulphur, though this solution would be dependent on the rate of pyrites oxidation at high pH values being slow enough to eliminate the formation of significant amounts of acid during a single dry season. That this approach might be practicable given suitable conditions, is borne out by the results of Harmsen *et al.*, who found high concentrations of pyrites sulphur in reclaimed soils which had been under normal cultivation for extremely long periods.

With these problems in mind it will be necessary to carry out further investigations on the way in which pyrites are formed and oxidised in the soils. The present results give no information on the mechanism by which pyrites oxidation proceeds, except in confirming that low pH values are necessary, though even this is not certain as Harmsen *et al.* have described some soils of high calcium content in which pyrites oxidation proceeded relatively rapidly. It is still not certain how pyrites are initially attacked. Quispel *et al.* suggested an initial chemical splitting of the pyrites molecule with the formation of elemental sulphur in an analogous way to its formation from monosulphide (Verhoop c.f. Quispel *et al.* [12];

Wiklander *et al.* [23]). The elemental sulphur could then be oxidised by bacteria, the acid formed accelerating the decomposition of more pyrites. On the other hand, Temple and Delchamps [17] found that bacterial iron oxidation by *Thiobacillus ferro-oxidans* under acid conditions was important in the formation of acid from pyrites in disused bituminous coal mines. The ferric iron produced was said to react chemically with pyrites to release elemental sulphur from which acid could be formed, either by the action of *Thiobacillus thio-oxidans* or by a further chemical oxidation by ferric ions.

Although the present results contain no direct evidence that it was the pyrites fraction of the soil sulphur which remained unoxidised in the soil where the pH value was kept above 3, this seems very likely from the previous work on pyrites oxidation mentioned above. The results indicated that when pyrites sulphur was oxidised, the sulphate formed was insoluble. The decrease in bacterial activity which occurred below pH 3.0 indicates that sulphate formation below this pH value was not entirely due to the activity of sulphur-oxidising bacteria. The solution of ferric ions which also occurred below pH 3 enables the oxidation of the pyrites fraction to be explained by the chemical mechanism postulated by Temple and Delchamps, and it seems unlikely that the iron-oxidising bacteria were important in pyrites-oxidation under the experimental conditions. However, preliminary experiments have shown that these bacteria can be cultured from the soil, and iron oxidation in a soil percolation apparatus has been shown to be azide sensitive. It may be that bacterial iron oxidation is important in intensifying the acid conditions resulting from sulphur oxidation. This aspect of the work is receiving further study.

The inferences made here on the way in which pyrites are oxidised in the soil may apply only to conditions in which the soil acidity increases rapidly and reaches the critical pH value for the solution of ferric iron. In a soil which contained the bulk of its sulphur as pyrites, the rate at which the soil-pH value would be expected to fall would be reduced and the decomposition of polysulphides at higher pH values might be a slower process, possibly involving mechanisms such as have been proposed by Quispel *et al.* [12].

SUMMARY

Tidal mangrove soil contained about 17-mg/g (oven-dry soil) of oxidisable sulphur, of which about 9 mg was insoluble in acetone. Samples showed considerable variability and this was shown to be due to the fact that decayed wood in the soil was heavily impregnated with oxidisable sulphur, a high proportion of which was insoluble in acetone. It is suggested that this proportion was the polysulphide fraction.

When the soil was dried, its pH value fell to 3.0 to 2.4 due to the activity of sulphur-oxidising bacteria. When the pH value of the soil fell below 3 a rapid decline in the number of the organisms present occurred, and it is suggested that this was due to the increase in the availability of ferric iron which also occurred below this pH value.

$CaCO_3$ had two main effects on sulphur oxidation; one on the sulphur-oxidising bacteria, increasing or decreasing sulphur oxidation according to whether the pH value was moved into or out of their range of activity, and an inhibitory effect on pyrites oxidation. The results indicate that the pyrites fraction was not oxidised above pH 3 and that it was not involved in acid-formation. It is suggested that pyrites oxidation under the experimental conditions was a chemical reaction possibly involving ferric ions.

The possible application of the results to the reclamation of saline mangrove swamps is discussed.

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DECOMPOSITION OF FOREST LITTERS

I. PRODUCTION OF AMMONIA AND NITRATE NITROGEN, CHANGES IN MICROBIAL POPULATION, AND RATE OF DECOMPOSITION *

by K. C. IVARSON and F. J. SOWDEN

Canada Department of Agriculture, Ottawa

INTRODUCTION

In 1878 Müller of Denmark recognized two types of humus formation in forest soils and named these "mull" and "mor" [14]. The mull type of humus has a loose, friable, crumbly structure; the organic matter is well mixed with the mineral soil and plant residues can no longer be distinguished. In the case of the mor type of humus, plant residues can still be recognized and organic matter remains on the surface of the mineral soil [14]. Soils developed under a well-drained mor have a podzol profile whereas soils under a well-drained mull have a brown earth profile [12].

Different opinions exist concerning factors affecting development of mull and mor. According to Handley [6] many investigators regard vegetation as an important factor. The mull type is usually found under deciduous litter, whereas the mor type is usually found under coniferous litter. The reasons for this are not well established, although it is believed that difference in the chemical composition of the litter may be partly responsible. Broadfoot and Pierre [2] believe that the over-riding factor governing the type of humus produced is the rate of decomposition of the litter, depending largely upon the inorganic fraction of the dead leaves. Many workers have concluded that climate exerts a primary control, more particularly in the case of mor, where low temperatures

* Joint contribution from the Divisions of Bacteriology (No. 469) and Chemistry (No. 412), Science Service.

depress rate of decomposition [6]. Romell [11] suggests that the type of humus formation is determined by types of micro-organisms present. Mull is dominated by bacteria, whereas in mor fungi play the dominant role.

As the result of a recent investigation Handley [6] has suggested that stabilized leaf proteins play an important part in mor formation. The mesophyll tissues of mor-forming litter contain stabilized protein which is generally very resistant to microbial attack. The vascular tissues of these same leaves do not appear to contain stabilized protein, hence the vascular tissues decompose leaving a residue of leaf mesophyll cell walls. Although stabilized protein may occur in mull-forming litter, Handley [6] believes this is not as resistant to decomposition as that of the mor-forming litter. He infers that greater resistance to transformation by mor-forming litter is probably the reason for the presence of recognizable plant residues in mor soils.

The object of the investigations reported here was to study the following processes in decomposing mull- and mor-forming types of litter: (a) production of ammonia nitrogen and nitrate nitrogen; (b) comparative changes in microbial population; (c) rate of decomposition; (d) changes in protein and hexosamine constituents. It was hoped that such work might supply information that would help account for the difference in the formation of mull and mor.

MATERIALS AND METHODS

Litter material

The samples of litter used in this experiment were gathered in May, 1957. The leaves were ground in a Wiley mill to pass through a 20-mm screen. The pH of the litters was determined with a glass electrode.

The following litters were used for this experiment:

A. A mixture of deciduous leaves consisting of approximately 80 per cent maple, 15 per cent beech and 5 per cent oak; 1.12 per cent N, pH 4.60.

B. A coniferous litter composed of white-pine needles only; 0.75 per cent N, pH 4.40.

Soils

The following soils were collected from the upper humus layer of the profile and ground to pass through a 2-mm screen:

A. A brown forest soil found in the vicinity of Ottawa, 0.81 per cent

N, pH 7.00. The soil had a typical mull characteristic and had developed under the stand of deciduous trees, the leaves of which were used in the experiment.

B. A podzol soil found approximately 25 miles north of Montreal, 0.86 per cent N, pH 4.40. The humus layer consisted of a fibrous mor, partially decomposed and had developed under the stand of white pine trees, the needles of which were used in the experiment.

Experimental procedure

The various cultures used in these studies are described below:

1. Podzol soil.
2. Brown forest soil.
3. Coniferous litter–podzol soil.
4. Deciduous litter–brown forest soil.
5. Coniferous litter–podzol soil–antifungal antibiotic.
6. Coniferous litter–podzol soil–antibacterial antibiotic.

Cultures number 1 and 2 consisted of 25 g of soil (oven-dry basis). In the case of the other cultures 75 g of ground litter (oven-dry basis) was added to 25 g of soil (oven-dry basis). After a thorough mixing, the materials were transferred to 1000-ml Erlenmeyer flasks and distilled water added to bring the moisture content to 60 per cent water-holding capacity. The flasks were plugged with cotton and incubated at 28° C, for a period of six months. The moisture content was maintained within narrow limits throughout the experiment by addition of water when necessary.

Groups 1 to 4 were duplicated and four series prepared in order to make the following determinations periodically:

a. Carbon dioxide evolution.
b. Numbers of bacteria, actinomycetes, and fungi.
c. Total N, ammonia-N, nitrate-N, hexosamines, and dry matter.
d. Amino acids.

In an attempt to retard growth of fungi 1 per cent cycloheximide (85–100 per cent active ingredient) was added to number 5. To bring about suppression of bacteria 1 per cent streptomycin (780 μg dihydrostreptomycin base per mg) and 0.5 per cent chloramphenicol (U.S.P) were added to number 6. The coniferous litter–podzol soil mixtures were treated with antibiotics because of the widely held view that fungi are the most important microbial agents in the formation of mor [6 12]. It seemed possible that antibiotics might suppress bacterial and fungal activity and help clarify this problem.

The pH of the deciduous litter–brown forest soil mixtures was adjusted to neutrality by the addition of 1 per cent calcium oxide. Calcium oxide was added to these mixtures because numerous investigators [6 12] have found generally that mull occurring under deciduous forest tends to have a neutral reaction.

Biological and chemical analyses of samples

Total N was determined by the Kjeldahl method. The amounts of am-

monia-N and nitrate-N were determined by a modification of Mason's [8] procedure *. Dry matter was determined by drying weighed samples overnight at 110° C.

Numbers of bacteria, actinomycetes and fungi were determined at 4-day intervals during the first 16 days of incubation. Counts were made again on the 25th day. After 25 days numbers were determined at 2-week intervals and after 3 months of decomposition, at monthly intervals. At each sampling time quintuplicate plates of three dilutions were made. The numbers of bacteria and actinomycetes were determined by plating on soil-extract agar medium. The relative abundance of fungi was obtained by plating on rose bengal streptomycin agar [7]. All counts have been expressed on the basis of oven-dried material.

The quantity and rate of carbon dioxide evolved was determined by fitting flasks to a gas train from which they received air freed of CO_2. Carbon dioxide evolved from the cultures was trapped in a $Ba(OH)_2$ solution and measured periodically by titration with oxalic acid. Results have been calculated as carbon dioxide-carbon.

RESULTS

As can be seen from Table 1, liberation of nitrogen in the form of ammonia occurred somewhat more rapidly in decomposing deciduous litter–brown forest soil mixture than in that of the coniferous litter–podzol soil mixture. Towards the end of the experiment nitrification began in the deciduous litter–brown forest soil mixture but was absent in the coniferous litter–podzol soil mixture. Table 1 shows also that in both of the decomposing litter mixtures percentage total nitrogen increased as dry matter decreased. The results also indicate that no significant loss of nitrogen as ammonia occurred, because if corrections are made for loss of dry matter, total nitrogen remains constant.

The results of the microbial counts are plotted in Figs. 1 to 8. In all cases it was found that the numbers of actinomycetes were about one-tenth those of bacteria, therefore, numbers of bacteria and actinomycetes were combined (as shown in Figs. 1, 3, 5, 7). Furthermore, it should be noted that in Figs. 1 and 5 microbial numbers have been expressed in billions per gram, whereas in Figs. 2 to 4 and 6 to 8 the results are in millions per gram. Comparing litters with added soil (Figs. 1 and 2) the bacteria and actinomycetes

* For details of this procedure (to be published) we are indebted to Dr. H. T. Tribe, School of Agriculture, Cambridge, England.

TABLE 1

Nitrogen and dry-matter changes in the decomposing litter mixtures

Decomposition (days)	Culture No.	Nitrogen (%)	NH_4-N (mg/g)	NO_3-N mg/g)	Dry matter (%)
0	3	0.79	N.D. *	N.D. *	100.0
	4	1.01	,,	,,	100.0
	5	0.82	,,	,,	100.0
	6	0.95	,,	,,	100.0
25	3	0.81	,,	,,	93.4
	4	1.06	,,	,,	91.7
	5	0.89	,,	,,	93.3
	6	1.03	,,	,,	93.0
53	3	0.80	— **	— **	91.8
	4	1.07	—	—	89.5
	5	0.91	—	—	92.1
	6	1.04	—	—	90.0
81	3	0.86	0.02	—	89.2
	4	1.19	0.04	—	84.5
	5	0.94	0.11	—	88.1
	6	1.05	0.06	—	88.5
137	3	0.89	—	—	87.0
	4	1.40	0.16	0.06	76.6
	5	0.95	—	—	86.1
	6	1.04	0.11	—	86.0
165	3	0.96	0.02	—	85.0
	4	1.46	0.16	0.09	72.4
	5	1.04	0.08	—	85.2
	6	1.11	0.10	—	85.0

* N.D. not determined.

** NH_4- and NO_3-N in trace amounts or less.

were consistently more numerous in the deciduous litter–brown forest soil mixture than in the coniferous litter–podzol soil mixture; in the case of fungi the reverse occurred. It is also obvious that this same trend exists in soils without added litters (Figs. 3 and 4).

Figures 5 and 6 show the results of adding antibiotics to the coniferous litter–podzol soil mixture. The antifungal antibiotic (cycloheximide) has little or no effect on numbers of fungi while the antibacterial antibiotics (streptomycin and chloramphenicol) reduced only slightly the numbers of bacteria and actinomycetes. An interesting feature is the large number of bacteria and actino-

mycetes in the cultures receiving the antifungal antibiotic and the apparent stimulation of fungal growth where antibacterial antibiotics were added. Periodic checks on the pH of the decomposing mixtures showed very little change in the reactions of all litter–soil

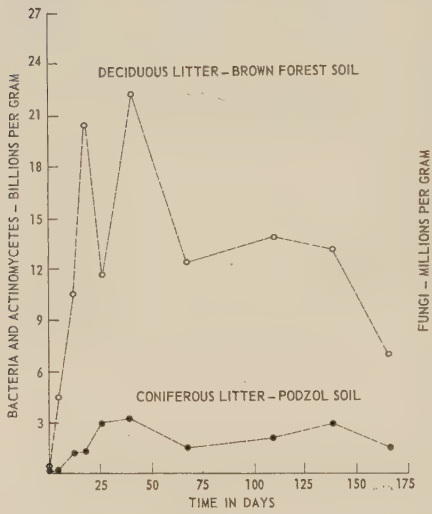
BACTERIA AND ACTINOMYCETES – BILLIONS PER GRAM
DECIDUOUS LITTER – BROWN FOREST SOIL
CONIFEROUS LITTER – PODZOL SOIL
TIME IN DAYS

Fig. 1

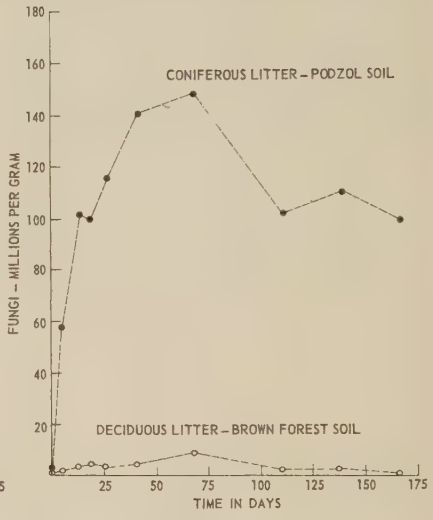

Fig. 2

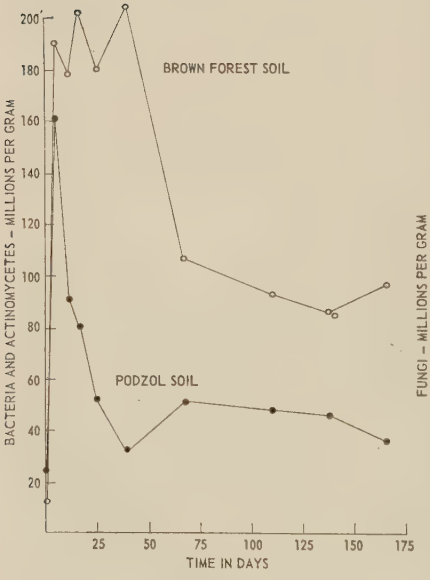

Fig. 3

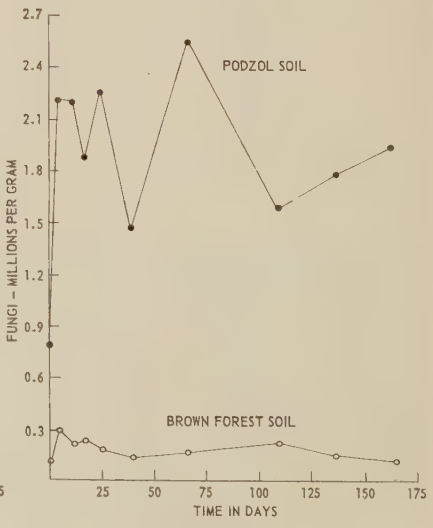

Fig. 4

mixtures, hence changes in microbial population cannot be attributed to change in pH.

Later investigations included studies of the effect of antibiotics on microbial development in the podzol soil without added litter. The antibiotics were added at the same rate as that of the litter–soil mixture. The numbers of micro-organisms were determined periodically for a period of 39 days. The results (Figs. 7 and 8) are in good agreement with those of Figs. 5 and 6, with the exception that a greater decline in numbers of fungi occurred where antifungal antibiotic was added.

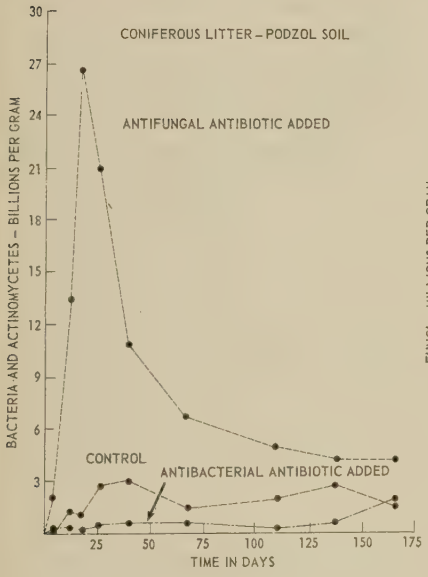

Fig. 5

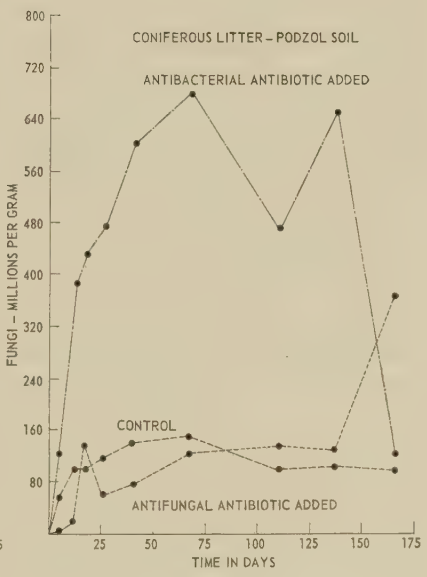

Fig. 6

The rate of evolution of carbon-dioxide carbon is presented in Fig. 9. The podzol soil without added litter showed greater activity than the brown forest soil. At the end of the experiment 57 per cent more carbon was lost from the podzol soil than from the brown forest. This is probably due to the fact that the organic residue in the podzol soil is only partially decomposed. The deciduous litter–brown forest soil mixture decomposed more rapidly than the coniferous litter–podzol soil mixture.

Rate of decomposition appears to have been increased slightly

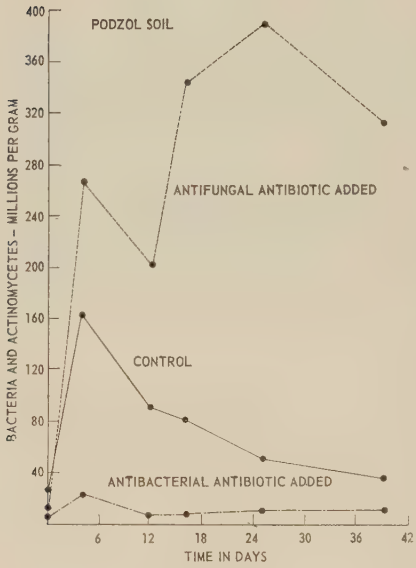

Fig. 7

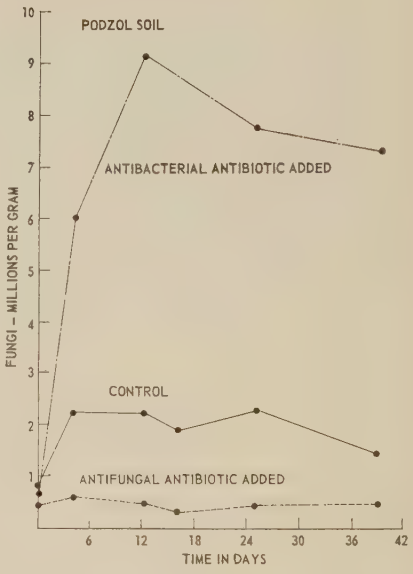

Fig. 8

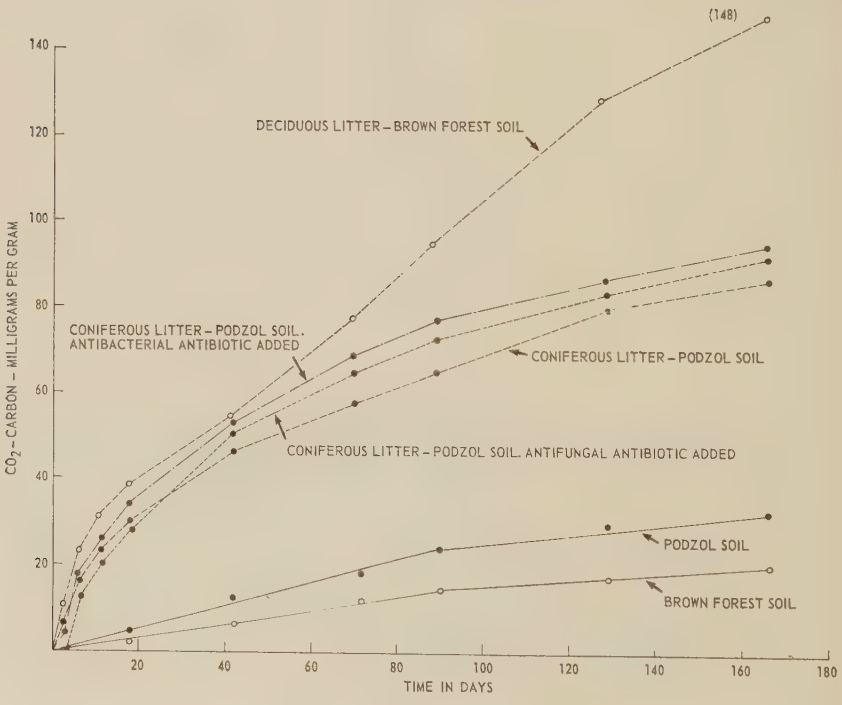
(148)
DECIDUOUS LITTER – BROWN FOREST SOIL
CONIFEROUS LITTER – PODZOL SOIL. ANTIBACTERIAL ANTIBIOTIC ADDED
CONIFEROUS LITTER – PODZOL SOIL
CONIFEROUS LITTER – PODZOL SOIL. ANTIFUNGAL ANTIBIOTIC ADDED
PODZOL SOIL
BROWN FOREST SOIL
CO_2 – CARBON – MILLIGRAMS PER GRAM
TIME IN DAYS

Fig. 9

by the addition of antibiotics to the coniferous litter–podzol soil mixture. After 88 days of incubation, 18 and 12 per cent more carbon was lost where antibacterial and antifungal antibiotics were added respectively. It should be mentioned that during the first 48 hours of incubation a decrease in CO_2 production was observed with both mixtures receiving antibiotics; this was very noticeable in the case of the antifungal compound.

DISCUSSION

In the studies made it was observed that nitrate formation occurred in the decomposing deciduous litter mixture but was absent from the decomposing coniferous litter mixture. It is possible that the differences observed between the litters is due to the larger quantity of total N present in the deciduous litter mixture. The mull types of soil may also provide conditions which are more favorable for nitrification; mor soils formed under coniferous forest covers are acid and the evergreen leaves contain tannins, resins, and terpenes which are toxic to nitrifying micro-organisms [14].

Microbial counts showed that bacteria and actinomycetes were consistently less numerous in both the mor soil and the decomposing coniferous litter mixture than in the mull soil and the decomposing deciduous litter mixture; in the case of fungi the reverse occurred. From a knowledge of the influence of acidity upon bacterial development it might be expected that soils and litters of coniferous forests would contain fewer bacteria and actinomycetes than soils and litters of deciduous forests. The work of Chase and Baker [3], and Vandecaveye and Katznelson [13] suggests that the nature of the organic residues could be responsible for this selective action.

The observed shift in the population brought about by the addition of antibiotics to the coniferous litter–podzol soil mixture is of interest. Pramer and Starkey [9] found that streptomycin added to soil at the rate of 1,000 ppm reduced slightly numbers of bacteria for the first week; this was followed by an increase in numbers after 4 to 6 weeks. When perfused over 30 g of soil chloramphenicol at a concentration of $M/1{,}300$ inhibited nitrite-oxidizing organisms for a period of 10 days [10]. In the present experiment it appears that the addition of antibacterial antibiotics somewhat

reduced numbers of bacteria and actinomycetes for a period of 137 days. The prolonged effect of the antibiotics could be due to the high concentrations of antibiotics employed (10,000 ppm streptomycin and 5,000 ppm chloramphenicol). Also, Bigger [1] has reported that these two antibiotics used in combination exhibit a high degree of synergisms.

The antifungal antibiotic increased the numbers of bacteria and actinomycetes and the antibacterial antibiotics increased the numbers of fungi. It is possible that the bacteria and actinomycetes were permitted to increase when competitive fungi were suppressed, the reverse occurring when the bacteria were reduced. However, Gottlieb [4], Pramer and Starkey [9], and Waksman and Woodruff [15] have shown that antibiotics added to soil are decomposed, hence any increase in numbers of micro-organisms may have resulted from the decomposition of the antibiotics. Furthermore, the results of Grossbard [5] indicate that some antibiotics may stimulate spore formation in fungi; such effects, if any, may account for a higher fungal count where antibacterial antibiotics were used.

It is evident from the results obtained in this investigation that mull-forming litters decompose faster than mor-forming litters. But the data give no indication as to what factors affect decomposition rate. The difference in decomposition rate may be due to higher base content of the deciduous leaves [2], which in turn might control the nutrient supply of the micro–organisms. Furthermore, several workers [12] have shown that wide variations in lignin and wax content of plant material markedly influence rate of decomposition. For a more detailed discussion regarding factors affecting rate of litter decomposition the authors refer the reader to Handley's [6] comprehensive study which contains an excellent literature review.

Since antibiotics are known to be susceptible to microbial attack in soil [4 9 15], it is possible that the small increase observed in the decomposition of the coniferous litter–podzol soil mixture with added antibiotics was the result of the microbial degradation of the antibiotics and not due to an increase in the rate of decomposition of coniferous litter. If this is so, it would suggest that the observed change in microbial population caused no acceleration in the decomposition of the coniferous litter. This supposition would imply that

the type of microbial population is not as important as the type of litter or some other factor in regard to decomposition rate.

The general impression to be derived from the experimental data presented is that litter is one of the important factors affecting development of mull and mor. The reason for this is that under the conditions of the experiment, the mull-forming litter had a tendency to decompose faster than the mor-forming type. While no attempt has been made in this part of the project to correlate changes in organic constituents with litter decomposition, the subject appeared worthy of study and is reported in a subsequent paper.

SUMMARY

Studies have been made on the production of ammonia and nitrate nitrogen, rate of decomposition along with comparative changes in microbial population during decomposition of deciduous and coniferous litters. At the same time the effect of addition of antibiotics on the microbial population and rate of decomposition of coniferous litter was investigated.

Liberation of nitrogen in the form of ammonia occurred somewhat more rapidly in decomposing deciduous litter than in that of the coniferous litter. Towards the end of the experiment the nitrification process commenced in the deciduous litter but was absent in the coniferous litter.

The deciduous litter decomposed more rapidly than the coniferous litter.

The bacteria and actinomycetes were consistently more numerous in the deciduous litter than in the coniferous litter; in the case of fungi the reverse occured. An antifungal antibiotic (cycloheximide) had little or no effect on numbers of fungi in the decomposing coniferous litter while antibacterial antibiotics (streptomycin and chloramphenicol) reduced slightly the numbers of bacteria and actinomycetes. Numbers of bacteria and actinomycetes were increased greatly in cultures receiving the antifungal antibiotic and fungal growth was apparently stimulated where antibacterial antibiotics were added. In spite of the observed shift in the microbial population brought about by the addition of antibiotics the rate of decomposition of the coniferous litter appeared to be unaffected. This would suggest that the type of microbial population is not as important as the type of litter or some other factor in regard to decomposition rate.

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DECOMPOSITION OF FOREST LITTERS

II. CHANGES IN THE NITROGENOUS CONSTITUENTS *

by F. J. SOWDEN and K. C. IVARSON

Canada Department of Agriculture, Ottawa

INTRODUCTION

A number of studies of the amino–acid composition of soil hydrolysates have been made [2 19 22] and it appears that there are no very distinctive differences in the amino-acid distribution between different organic fractions from soils [3 4 17], between soils or between different soil horizons [20]. However, there are no quantitative data to show changes in the amino-acid composition of different forest litters during their decomposition. Bremner [5] found little qualitative differences in decomposing straws, but Rothwell and Frederick [18] found some changes in the amino-acid composition of alfalfa and corn stover decomposing at different temperatures.

The hexosamine content of a number of soils and soil profiles has been studied [6 23] and Bremner [5] has found an increase in the hexosamine content of decomposing straws.

This part of the present project was undertaken to follow the changes, if any, in the amino-acid and hexosamine content of decomposing pine needles and deciduous leaves to determine if any changes took place in the content of these substances at relatively early stages in the decay of forest litters and to see if any of these changes shed any light on the formation of mull and mor.

MATERIALS AND METHODS

The materials used in this investigation have been described in Part I [15] as have the methods of composting and sampling.

* Joint contribution from the Divisions of Chemistry (no 413) and Bacteriology (no 470), Science Service.

The Moore and Stein method [16] for amino-acid analysis as modified for soils [19] was used. Hexosamines were determined by the Elson and Morgan [11] method as modified for soils by Stevenson [23] and in some instances by the method described by Eastoe [9].

RESULTS

Table 1 shows the amount of amino acids in the original soils and leaves and in the composts after mixing and wetting. The agreement between the individual mixtures and between their average and the amount they should contain as calculated from the amino-acid content of the leaves and soils gives a measure of the total error of the methods, including sampling and moisture correction. The range is seldom over ten per cent and is usually much less. The missing values (valine and methionine and isoleucine) were estimated by difference for calculating the total amino-acid nitrogen of the soils. It is seen that with the coniferous litter–podzol soil mixture, the soil supplied about $^1/_4$ of the amino acids; with the deciduous litter–brown forest soil mixture, the soil supplied only $^1/_6$ to $^1/_5$ of this total. In each instance – as was to be expected – the percentage of the total nitrogen contained in the basic amino acids, the acidic amino acids and the total amino acids was lower in the soil than in the leaves. There was no very distinctive difference between the two soils or between the two litter materials in these percentages. The percentage of the total amino-acid nitrogen represented by the α-amino nitrogen of the individual amino acids is also shown. This gives a measure of the molar ratios of the amino acids in the different materials.

Table 2 shows that as carbon was lost from the decomposing soil–litter mixtures there was a gradual increase in the amount of amino acids per gram of sample except perhaps for methionine and tyrosine with the coniferous litter compost. This increase may be slightly greater, when the amount of acid present is considered, for aspartic acid, glutamic acid, glycine, alanine and leucine than for most of the other amino acids especially with the deciduous leaves–brown forest soil compost; there was also a rather large percentage increase in the histidine content of the coniferous leaves–podzol soil compost but not with the deciduous leaves mixture. With both mixtures there appeared to be an increase in

TABLE 1

Amino-acid composition of decomposing litter–soil mixtures (in mg)

Amino acid	1 g podzol soil	3 g coniferous litter	4 g coniferous litter–podzol soil mixture calc.	4 g coniferous litter–podzol soil mixture			α-amino N/tot. N		1 g brown forest soil	3 g deciduous litter	4 g deciduous litter–brown forest soil mixture calc.	4 g deciduous litter–brown forest soil mixture			α-amino N/tot. N	
				1	2	ave	coniferous litter	podzol soil				1	2	ave.	deciduous litter	brown forest soil
Aspartic acid	3.92	12.98	16.90	16.36	15.29	15.83	9.6	9.9	4.30	16.91	21.21	23.70	21.19	22.45	8.3	12.7
Threonine	2.27	6.84	9.11	9.56	9.47	9.53	5.6	6.4	1.91	9.59	11.50	11.77	12.22	11.99	5.2	6.3
Serine	2.06	6.74	8.80	9.29	8.24	8.77	6.3	6.6	1.66	9.76	11.42	12.19	11.56	11.88	6.0	6.2
Glutamic acid	3.21	11.75	14.96	15.38	15.53	15.46	7.9	7.4	2.83	19.79	22.62	21.82	22.89	22.36	8.8	7.6
Proline	1.52	6.00	7.52	7.01	7.50	7.26	5.1	4.5	1.17	8.99	10.16	10.23	9.49	9.86	5.1	4.0
Glycine	2.41	6.62	9.03	10.49	9.23	9.86	8.7	10.8	2.42	9.59	12.01	12.63	12.40	12.52	8.3	12.7
Alanine	2.31	7.39	9.70	8.89	9.10	9.00	8.1	8.8	2.08	10.09	12.17	12.77	11.91	12.34	7.4	9.2
Valine	—	5.89	—	9.51	9.02	9.26	4.6	—	—	10.17	—	13.44	11.17	12.31	5.7	—
Methionine	—	—	—	2.03	2.21	2.12	—	—	—	2.44	—	3.35	3.14	3.25	1.1	—
Isoleuoine	—	6.16	—	7.60	7.93	7.77	4.6	—	1.30	9.12	10.42	11.17	10.30	10.74	4.5	3.9
Leucine	2.53	10.44	12.97	13.20	13.04	13.12	7.8	6.5	1.75	14.88	16.61	15.75	16.86	16.31	7.4	6.2
Tyrosine	0.74	3.49	4.23	4.77	5.23	5.00	1.9	1.4	0.36	5.30	5.66	5.58	5.84	5.71	1.9	0.8
Phenylalanine	1.38	6.71	8.09	7.85	8.43	8.14	4.0	2.8	1.06	8.32	9.38	11.17	9.77	10.47	3.3	2.5
Histidine	0.98	1.42	2.40	2.51	1.70	2.10	0.9	2.1	0.54	3.22	3.76	3.09	3.50	3.29	1.3	1.4
Lysine	1.56	6.58	8.14	8.18	7.80	7.99	4.4	3.6	1.15	10.45	11.60	11.04	11.11	11.08	4.6	3.1
Arginine	1.35	5.81	7.16	7.69	7.54	7.62	3.5	2.6	1.01	9.23	10.24	10.23	9.62	9.93	3.4	3.0
Amino acid-N % / Total-N	48	62							44	64						
Basic amino-N % / Total-N	12	18							9	17						
Acidic amino-N % / Total-N	8	11							9	11						

TABLE 2

Amino-acid composition of decomposing litter–soil mixtures (mg amino acid/g sample, oven-dry basis)

Mixture	Coniferous litter–podzol soil mixture						Deciduous litter–brown forest soil mixture						Coniferous litter–podzol soil–antifungal antibiotic mixture		Coniferous litter–podzol soil–antibacterial antibiotic mixture	
No. of days	0	25	53	81	137	165	0	25	53	81	137	165	53	137	53	137
Aspartic acid	3.96	4.37	4.38	4.46	4.71	4.97	5.61	5.80	6.10	7.19	7.44	8.04	4.68	4.83	4.31	4.86
Threonine	2.38	2.47	2.61	2.83	3.07	2.92	3.00	3.31	3.38	4.02	3.98	4.32	2.77	2.99	2.39	2.97
Serine	2.19	2.40	2.25	2.74	2.62	2.64	2.97	3.33	3.26	3.47	3.62	3.83	2.39	2.69	2.40	2.64
Glutamic acid	3.84	4.29	4.26	4.57	4.46	4.75	5.59	6.40	7.62	6.75	7.01	7.80	4.33	5.16	4.22	4.27
Proline	1.81	1.97	2.09	2.04	2.12	2.34	2.47	2.92	2.84	3.16	3.55	3.80	2.21	2.09	2.20	2.09
Glycine	2.47	2.41	2.57	2.63	2.83	3.11	3.13	3.48	3.57	3.87	4.26	4.87	2.73	2.84	2.73	2.94
Alanine	2.25	2.69	2.77	2.80	2.79	3.23	3.09	3.47	3.81	3.55	4.58	4.48	2.87	3.21	2.86	2.66
Valine	2.35	2.50	2.65	2.33	2.42	2.77	3.08	3.28	3.59	3.83	3.88	4.18	2.79	2.83	2.63	2.71
Methionine	0.53	0.64	0.69	0.60	0.60	0.36	0.81	0.93	0.95	0.73	0.93	0.92	0.71	0.66	0.71	0.53
Isoleucine	1.94	2.21	2.36	2.18	2.37	2.42	2.68	2.95	3.04	3.00	3.08	3.09	2.47	2.44	2.52	2.31
Leucine	3.28	3.55	3.79	3.89	3.94	4.13	4.08	4.85	5.06	4.77	5.28	5.09	4.11	4.10	4.01	4.04
Tyrosine	1.25	0.96	1.14	1.26	1.32	1.22	1.43	1.51	1.27	1.55	2.10	1.75	1.24	1.06	1.41	1.01
Phenylalanine	2.04	2.07	2.25	2.41	2.33	2.50	2.62	2.71	3.00	2.91	3.14	3.19	2.45	2.39	2.51	2.53
Histidine	0.53	0.67	0.76	0.59	0.78	0.96	0.82	0.90	0.78	0.74	0.93	0.97	0.87	0.84	0.83	0.61
Lysine	2.00	1.89	2.01	2.35	2.20	2.06	2.77	2.67	2.51	2.79	2.81	3.04	2.09	2.22	2.08	1.85
Arginine	1.90	1.88	2.12	1.96	2.02	2.07	2.47	2.52	2.49	3.12	2.67	2.70	2.07	2.08	1.89	1.94
$\frac{\text{Amino-N}}{\text{Total-N}}$ %	60	62	65	62	61	60	63	65	66	63	56	56				
$\frac{\text{Basic-amino-N}}{\text{Total-N}}$ %	14	14	16	14	14	14	15	15	14	15	12	12				
$\frac{\text{Acidic-amino-N}}{\text{Total-N}}$ %	10	11	11	11	10	10	11	12	13	12	10	11				

the amount of total nitrogen accounted for as amino-acid nitrogen followed by a decrease toward the end of the experiment.

In Table 3 is shown the molar ratios of the amino acids found at the different sampling dates. These data were calculated on the basis of the α-amino nitrogen of each amino acid divided by the total amino-acid nitrogen and multiplied by 100. There were no definite trends in the data, except possibly for a decrease in the methionine and an increase in the histidine ratios with the decomposing coniferous litter–podzol soil mixture. There were, however, frequent instances where the change in an amino acid ratio was greater than the usual 10 per cent range.

The changes in the hexosamine content of the decaying litter–soil mixtures are shown in Table 4. The method described by Stevenson was employed for most of the determinations and the factor he proposed was used to correct for loss on hydrolysis since this factor was similar to the one used by Bremner [6] and it was felt that it would give comparative data through the experiment. When the hydrolysates were being analyzed for basic amino acids by the method of Moore and Stein, two peaks were found before the appearance of histidine. These peaks occupied the same position as glucosamine and galactosamine in the elution curves and had the same R_F values in butanol–acetic acid–water (4 : 1 : 1). Glucosamine also had the same R_F value in pyridine–ethyl acetate–water–acetic acid (5 : 5 : 3 : 1) [13] as had the material in the first peak. The amount of galactosamine recovered from the columns was low and its identification with this solvent system was doubtful The shape of the two peaks and the paper chromatograms gave no evidence that more than two compounds were present. A search of the literature revealed that Eastoe [9] had proposed using the Moore and Stein basic columns and buffer systems for the quantitative determination of these two amino sugars. Experiments with added glucosamine showed that 50 per cent was destroyed during hydrolysis for amino-acid analysis with the pine–needle compost and more than 60 per cent with the deciduous compost. Hence the total hexosamine of the coniferous and deciduous composts as determined by the Eastoe method was multiplied by 2 and 2.5 respectively for comparison with data obtained by the Elson and Morgan method.The agreement was fairly good with coniferous litter but not with the deciduous leaves.

TABLE 3

Molar ratios of amino acids (Calculated on the basis of 100 × α-amino-N of each acid/total-N of amino acids)																
Mixture	Coniferous litter–podzol soil mixture						Deciduous litter–brown forest soil mixture						Coniferous litter–podzol soil–antifungal antibiotic mixture		Coniferous litter–podzol soil–antibacterial antibiotic mixture	
No. of days	0	25	53	81	137	165	0	25	53	81	137	165	53	137	53	137
Aspartic acid	8.9	9.2	8.3	8.8	9.1	9.1	9.4	8.9	9.1	10.1	10.0	10.3	9.0	8.9	8.5	9.6
Threonine	6.0	5.8	5.9	6.3	6.6	6.0	5.6	5.7	5.6	6.4	6.0	6.0	6.0	6.2	5.3	6.6
Serine	6.2	6.4	5.7	6.9	6.4	6.1	6.3	6.5	6.4	6.2	6.1	6.1	5.8	6.3	6.0	6.6
Glutamic acid	7.9	8.2	7.7	8.2	7.8	7.9	8.5	8.9	10.3	8.6	8.5	9.0	7.5	8.6	7.6	7.6
Proline	4.7	4.9	4.8	4.7	4.7	5.0	4.8	5.2	4.9	5.2	5.5	5.6	4.9	4.5	5.0	4.8
Glycine	9.9	9.0	9.1	9.2	9.7	10.1	9.3	9.5	9.4	9.7	10.1	11.0	9.3	9.3	9.6	10.3
Alanine	7.6	8.5	8.3	8.3	8.0	8.9	7.7	8.0	8.5	7.5	9.2	8.5	8.2	8.7	8.5	7.8
Valine	5.9	6.0	6.1	5.2	5.3	5.8	5.8	5.7	6.1	6.1	5.9	6.0	6.1	6.0	5.9	6.1
Methionine	1.1	1.2	1.2	1.0	1.0	0.6	1.2	1.3	1.3	0.9	1.1	1.0	1.2	1.1	1.2	1.0
Isoleucine	4.4	4.8	4.8	4.4	4.7	4.5	4.6	4.6	4.6	4.3	4.2	4.0	4.8	4.6	5.1	4.6
Leucine	7.5	7.6	7.7	7.9	7.7	7.7	6.9	7.6	7.7	6.8	7.2	6.6	8.0	7.7	8.0	8.1
Tyrosine	2.0	1.5	1.7	1.8	1.9	1.6	1.8	1.7	1.4	1.6	2.0	1.6	1.7	1.4	2.1	1.5
Phenylalanine	3.7	3.6	3.7	3.8	3.6	3.7	3.6	3.4	3.6	3.3	3.4	3.3	3.8	3.6	4.0	4.0
Histidine	1.0	1.2	1.3	1.0	1.3	1.5	1.2	1.2	1.0	0.9	1.1	1.1	1.4	1.3	1.4	1.0
Lysine	4.1	3.7	3.7	4.2	3.9	3.4	4.2	3.8	3.4	3.6	3.4	3.5	3.6	3.7	3.8	3.3
Arginine	3.3	3.0	3.3	3.0	3.0	2.9	3.2	3.0	2.8	3.4	2.7	2.6	3.0	2.9	2.8	2.9

TABLE 4

Hexosamine content of decomposing litter–soil mixtures

No. of days	Coniferous litter–podzol soil mixture					Deciduous litter–brown forest soil mixture					Coniferous litter–podzol soil–antifungal antibiotic mixture		Coniferous litter–podzol soil–antibacteria antibiotic mixture	
	Gluc	Galect.	Total × 2	E & M* Total Hex.	E & M* $\frac{\text{Hex-N}}{\text{Tot.-N}}$	Gluc.	Galact.	Total × 2.5	E & M* Total Hex.	E & M* $\frac{\text{Hex-N}}{\text{Tot.-N}}$	E & M* Total Hexosamine	E & M* $\frac{\text{Hex-N}}{\text{Tot.-N}}$	E & M* Total Hexosamine	E & M* $\frac{\text{Hex-N}}{\text{Tot.-N}}$
	mg/g	mg/g	mg/g	mg/g	%	mg/g	mg/g	mg/g	mg/g	%	mg/g	mg/g	mg/g	%
0	0.76	0.21	1.92	1.97	1.95	1.24	0.45	4.23	3.09	2.39	1.73	1.65	1.85	1.52
11	—	—	—	1.52	1.38	—	—	—	2.60	1.98	1.42	1.21	1.41	1.03
25	—	—	—	1.87	1.80	1.64	0.61	5.63	2.51	1.85	2.21	1.94	4.21	3.20
39	—	—	—	2.84	2.67	—	—	—	3.81	2.66	3.08	2.68	2.65	2.00
53	1.52	0.27	3.58	3.02	2.95	1.78	0.72	6.25	4.90	3.58	2.74	2.35	3.25	2.44
67	—	—	—	2.78	2.64	—	—	—	4.96	3.56	3.00	2.58	3.26	2.47
81	1.33	0.26	3.18	3.31	3.01	1.80	0.84	6.60	4.62	3.05	4.83	4.02	4.55	3.39
109	—	—	—	3.43	2.91	—	—	—	6.15	3.36	4.51	3.52	4.54	3.14
137	1.54	0.29	3.66	3.93	3.45	2.36	1.10	8.65	8.63	4.82	3.83	3.15	3.93	2.95
165	1.66	0.50	4.32	2.98	2.43	2.66	1.18	9.60	6.08	3.26	—	—	3.68	2.59
53 days with added antifungal antibiotic	1.47	0.36	3.66											
137 days with added antifungal antibiotic	1.55	0.41	3.92											

* E & M = Elson and Morgan method.

Both methods showed an increase in the hexosamine content of both materials during decomposition. There was a distinct difference between the two materials in the ratio of the two amino sugars: with the pine needles $^1/_6$ to $^1/_4$ of the total was galactosamine, with the deciduous leaves this proportion increased to $^1/_4$ to $^1/_3$. There was also a slight though perhaps not significant increase in the galactosamine content of the pine needles treated with the antifungal antibiotic. Streptomycin contains *n*-methyl glucosamine and it is not known what breakdown products it forms in soils. When it was used as the antibacterial antibiotic a single peak was found in the elution curves from the Moore and Stein columns. The identity of the material(s) in this peak is not known but calculated as glucosamine, it represented considerably more than half the amount of hexosamine found by the Elson and Morgan method.

DISCUSSION

As Table 1 shows, there was sometimes an extreme range of about 10 per cent between the amounts of individual amino acids found in samples from duplicate flasks; the same range was found in other duplicate data that are not reported. The usual range of values was considerably less than this but the fact that errors of this size were fairly common makes the interpretation of relatively small changes difficult. In many instances differences in the percentages of amino acids and in their ratios greater than this 10 per cent were found from one sampling date to another. But whether this represented a definite change in the composition of the protein-derived materials as the nitrogenous substances were broken down and synthesized cannot be definitely determined from these data. To secure unequivocal evidence on this point would require the analysis of a large number of samples at each date to permit the calculation of valid standard errors for each amino acid – a prohibitive amount of work at the present stage of amino acid analysis. Perhaps it should be said that there were no definitely demonstrable changes in the amino-acid composition of these materials during decomposition although the changes pointed out in the results may have some significance. Treatment of the coniferous litter–podzol

soil mixture with antibiotics had little apparent effect on the amino-acid composition.

It is tempting to assume that the small increase in total amino-acid nitrogen that is found in the first two months represents a net synthesis of protein by the micro-organisms but, considering errors involved in sampling and analysis, this increase may not be real. However, the errors in the determinations of the individual amino acids should tend to average out when the total amino–acid nitrogen is calculated and the increase occurred over the first two months with both soils. The same comment might apply to the decrease in amino-acid nitrogen that is found toward the end of the experiment; with the brown forest mixture the drop in the percentage of nitrogen accounted for would appear to be greater than the expected error.

When the materials (the two soils and leaves) were compared with the decomposing litters, it appeared that there were no very great differences between the leaves and the composts. However, the soils were definitely lower in amino-acid nitrogen than the composts. Also when their amino-acid ratios are compared with those of the composts, phenylalanine and perhaps arginine were low, and histidine and perhaps glycine high in the podzol soil, and aspartic acid and glycine were high, and tyrosine and leucine low in the brown forest soil.

The amino acids increased in absolute amounts on a percentage basis as decomposition proceeded, but this was largely the result of a loss of carbon from the sample. Toward the end of the experiment the percentage of total nitrogen represented by amino-acid nitrogen decreased. This is in accord with other results which indicate a decrease in amino nitrogen in more decomposed organic matter [5 20].

With the amino-acid-containing material there was no evidence of any significant amount of amino acids not usually found in proteins; no β-alanine or γ-aminobutyric was found, but small amounts might have escaped detection. There were usually two very small peaks between valine and methionine; one of these may have been α-ε-diaminopimelic acid. The amounts in these peaks – less than $^1/_4$ of the amount of methionine – were too small to permit any attempt at identification. Bremner [5] found little change in the amino-acid composition of decomposing straws, but did find small amounts of β-alanine and α-ε-diaminopimelic acid in a few instances.

There was no indication of the accumulation of amino acids found in bacterial cell-wall material. It had been shown that the cell walls of gram-positive bacteria are partly composed of peptide-like material containing lysine or α-ε-diaminopimelic acid, glutamic acid and alanine and occasionally glycine, serine, and aspartic acid [7 8 24]. The analysis failed to show any increase in the proportion of these amino acids. Stevenson [22] found a high ratio of lysine to arginine in some of the soils he studied, but there was no evidence of this with any of the materials analyzed in this study. Our results were also different from those of Rothwell and Frederick [18] who found changes in the amino-acid composition of alfalfa and corn stover decomposing at different temperatures.

Block and Weiss [1] have stated that the proteins in all living material have a similar composition and Fox and Homeyer [14] have made a statistical study of the amino-acid composition of a number of proteins and found a high degree of kinship among protein molecules. The amino-acid composition of the decomposing leaves fits into this picture; it is also similar to that of the soils analyzed previously [20] although the soil materials may have a slightly higher proportion of glycine and proline. There is thus no evidence for selective destruction of any amino acid during the formation of soil organic matter. Over a very long period of time, however, some selective destruction of amino acids apparently occurs since on very great ageing, *i.e.* in the formation of fossils, a change in the amino-acid composition takes place. The amino-acid content of bone [10] is not very different from that of other proteins but as the age increases some of the amino acids disappear while glutamic acid, aspartic acid, glycine, and sometimes a few others survive [12]. On the other hand, with soil materials if the amino-acid polymers are stabilized by union with other materials, *e.g.* by a browning reaction or similar combination, there is no selective loss of an amino acid such as arginine as was suggested previously [17].

The hexosamine content also gives no evidence for the accumulation of bacterial cell-wall material. No muramic acid was found, and evidently the sum of glucosamine and galactosamine by the Eastoe method accounted for all the hexosamine found by the Elson and Morgan method. This would appear to indicate that muramic acid was present in very small amounts, if at all, unless it was eluted with either glucosamine or galactosamine in such a way

as not to give a skewed elution peak and was not separated from them on butanol–acetic acid–water chromatograms. Most of the gram-positive bacteria have muramic acid and glucosamine in their cell–wall material [7 8]; a few have galactosamine also. Hence the source of the relatively large amounts of galactosamine found in this experiment is not known, although it does occur in the cell walls of some microbes.

The similarity in the amino-acid composition of the decomposing leaf litters would appear to indicate that changes in the composition of the proteins play little part in the formation of mull and mor. The relatively higher proportion of glucosamine in the total hexosamines of coniferous litter compost considered along with the higher relative numbers of fungi found therein might be an indication that fungal products are of relatively greater importance in mor formation.

SUMMARY

A study was made of the changes in the amino-acid and hexosamine content of coniferous litter–podzol soil and deciduous litter–brown forest soil composts during decomposition for a period of 165 days.

There was a gradual increase in the percentage of most of the amino acids (on an oven-dry basis) as carbon was lost from the system. The percentage of total nitrogen represented by the amino-acid nitrogen increased slightly for the first 53 days, then decreased; this decrease was greater with the deciduous litter compost.

There were some changes in the ratios of one amino acid to another (on a molar basis) that were greater than the expected error, but these changes formed no definite pattern and were more or less random, hence their significance is uncertain.

The hexosamine content also increased with time and this increase was greater than the carbon loss so that there was an increase in hexosamine nitrogen as a percentage of the total nitrogen throughout the duration of the experiment. More galactosamine relative to glucosamine was found in the deciduous litter compost and its total hexosamine content was greater than was that of the coniferous litter compost.

There appeared to be no changes in the amino-acid composition of the proteinaceous material in decomposing deciduous or coniferous litters that would help to explain the formation of mull and mor. Differences in the hexosamine content may indicate that bacterial products are of greater relative importance in deciduous litter than in coniferous litter.

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FURTHER OBSERVATIONS ON HUMUS DECOMPOSITION AND NITRIFICATION

by H. F. BIRCH

East African Agriculture and Forestry Research Organisation, Kikuyu, Kenya

INTRODUCTION

In a previous publication (Birch[2]) it was tentatively concluded that the flush of decomposition that follows the moistening of a dry soil was due to the high activity of the newly developing microbial population. Subsequent experiments to test this hypothesis have revealed (1) that microbial activity of the type described above follows each of a succession of treatments of a moist soil with certain organic vapours and that these treatments do not affect the solubility of the organic material, (2) that the flush of decomposition that follows the moistening of an air- or oven-dried soil is due to the effect of drying both on the amount of decomposable organic material that goes into solution and on the microflora, and (3) that this water-soluble material decomposes without the production of mineral nitrogen. These observations and the experimental evidence supporting them are discussed below.

Since successive flushes of decomposition were obtained by such varied successive treatments as dry heat, moist heat, cold drying, ether vapour, chloroform vapour, and formaldehyde vapour it was considered that a common factor, namely microbial death, was operative in each instance, removal of the adverse conditions being followed by a period of high activity associated with the newly developing population. It is now clear that the same effect can have different causes and recognition of this goes some way towards clarifying the problem of the mechanisms involved in humus decomposition, soil nitrification, and the partial sterilisation of soils.

EXPERIMENTAL

In the following experiments the amount of soil carbon mineralised was determined by diluting the caustic soda solution in the respirometer to 100 ml at the end of the experiment. Five ml were then titrated with $N/10$ hydrochloric acid against methyl orange (reading a). A second 5-ml aliquot was treated with excess 0.4 N barium chloride solution and titrated against phenolphthalein (reading b).

$$a\text{–}b \times 22.4 = \text{ml } CO_2 \text{ evolved}$$
$$a\text{–}b \times 12.0 = \text{mg carbon mineralised.}$$

To determine mineral nitrogen the soil was mechanically shaken for ½ hour with 50 ml $2N$ KCl, 20 ml N HCl and 30 ml H_2O (pH of extracting solution = 0.7). An aliquot of the filtrate (usually 50 ml) was then steam distilled after the addition of about 3 g of magnesium oxide. The ammonia was collected in 2% boric acid solution and the amount determined by titration with $N/70$ HCl, using a methyl red–methylene blue indicator. Devarda's alloy (2 g) was then added to the previously distilled extract and the distillation procedure repeated for the determination of nitrate nitrogen.

Experiment 1. The effect of charcoal on decomposition

Thirty-gram portions of soil (approx. 6% C and 0.6% N) which had been air dry for about 2 years were mixed with 0.0, 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 g of activated charcoal, brought to field capacity and put in the macro respirometer (Birch and Friend [3]) for 14 days (1). The samples were then oven dried for 48 hours, restored to field capacity and, without further addition of charcoal, again put in the respirometer for 14 days (2). This procedure was repeated once more (3).

The succeeding patterns of decomposition are shown in Fig. 1, while Table 1 shows the magnitude of decomposition for each of the 14-day periods.

TABLE 1

Amount of charcoal added (g)	0.0	0.5	1.0	1.5	2.0	2.5	3.0
mg soil C mineralised (1) *	45.0	34.8	30.6	27.6	24.0	25.8	22.8
,, ,, ,, ,, (2) *	46.2	34.2	27.6	24.0	23.4	22.2	21.6
,, ,, ,, (3) *	37.2	33.6	27.6	24.0	23.4	23.4	23.4

* See description of method.

In this experiment the charcoal adsorbs soluble decomposable material from solution (as can be demonstrated, by shaking, charcoal with an aqueous extract of dried soil), the degree of adsorption depending on the amount of charcoal added. Initially

1.5 g of charcoal was sufficient to absorb the bulk of the soluble material and the flush of decomposition was inhibited. With succeeding dryings and rewettings further amounts of organic material go into solution and the charcoal becomes increasingly saturated and less able to adsorb it completely. The flush of decomposition then gradually reappears with successive dryings and rewettings.

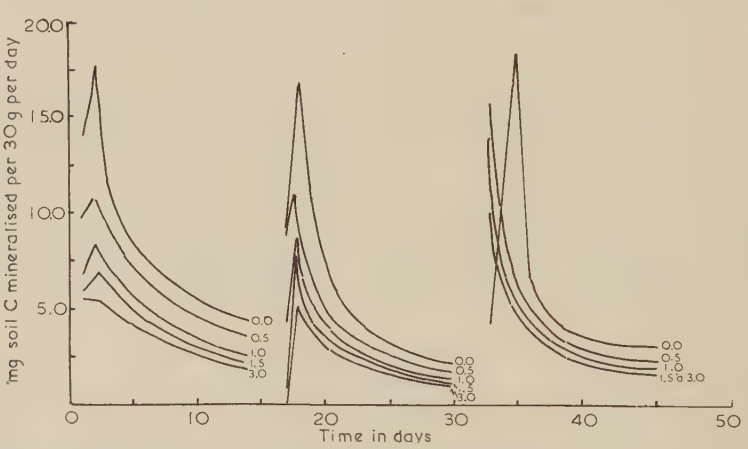


Fig. 1. Effect of activated charcoal on subsequent magnitudes of decomposition. (Numbers = g charcoal added. Graphs for 2.0 and 2.5 g charcoal omitted to avoid overcrowding).

Following treatment of a soil with ether vapour (Expt. 10) charcoal has no such depressive effect on the resulting flush of decomposition, nor does ether vapour affect the amount of organic material going into solution. This is evidence that the charcoal effect in Expt. 1 is due to the adsorption of soluble material and not to adsorption, and consequent inactivation, of the microbial population.

Experiment 2. The extraction of decomposable material

Two hundred grams of oven-dry soil (similar to that used in Expt. 1) were extracted for ½ hour (mechanical shaking) with 400 ml water (1). The soil was then filtered, using suction, and then immediately re-extracted with a further 400 ml water and filtered (2). This procedure was repeated four more times (3–6). The separate filtrates were concentrated to 50 ml and aliquots taken for the determination of ammonia nitrogen and nitrate nitrogen as well as total nitrogen (Kjeldahl's method). Since most of the nitrate nitrogen

is lost during the Kjeldahl digestion the difference between Kjeldahl nitrogen and ammonia nitrogen was taken as a measure of organic nitrogen. Organic carbon was determined by the Walkley-Black [14] method on an aliquot evaporated to dryness on the water bath. Other aliquots were reserved for decomposition studies.

The amounts of organic carbon and nitrogen per 50 ml of concentrate are given in Table 2.

TABLE 2

Extract No. *	mg NH_4-N	mg NO_3-N	mg Kjeldahl N	mg Org. N	mg Org. C	C/N
1	1.7	8.6	14.5	12.8	128.3	10.2
2	0.0	1.6	8.0	8.0	58.9	7.4
3	0.0	0.4	5.0	5.0	41.6	8.3
4	0.0	0.3	3.0	3.0	19.1	6.4
5	0.0	0.4	2.0	2.0	11.6	5.8
6	0.0	0.3	1.5	1.5	11.6	7.7

* See description of method.

Approximately 40% of the soluble material came out with the first extraction and progressively decreasing amounts thereafter. The C/N ratio of the extracted material is not dissimilar to that for soils in general.

To study the decomposition of the extracts 15–ml aliquots of the 50–ml concentrates were added to 40–g portions of dry soil similar to that used in Expt. 1 but taken direct from the field. As a control 40 g of soil were treated with 15 ml water. The samples were put in the respirometer for 10 days and the amounts of carbon mineralised and mineral nitrogen present were then determined (Table 3).

TABLE 3

Extract No.	1	2	3	4	5	6	Water
mg C mineralised	45.6	30.6	25.8	22.2	21.6	21.6	19.2
mg mineral N	6.21	4.19	3.43	3.86	3.93	4.28	4.26

The amounts of carbon mineralised were, in all instances, greater than in the control and the extra amounts were roughly proportional to the amounts of organic carbon in the extracts. In spite of this there is no evidence that this extra decomposition was accompanied by extra nitrogen mineralisation. The higher value for extract 1 was due to the greater amount of mineral nitrogen already present

in the added extract (see Table 2) compared with the other extracts and was not a consequence of the decomposition of organic nitrogen.

To confirm these observations 5-ml aliquots of each of the concentrated extracts were added to 50 g of medium–grade sand. 0.1 g of fresh soil was added as an inoculum and the samples put in the respirometer for 5 days after which the amounts of carbon mineralised and the amounts of mineral nitrogen present were determined. A control (sand + 5 ml water + 0.1 g soil) was included.

TABLE 4

Extract No.	1	2	3	4	5	6	Water
mg C mineralised	6.0	4.8	3.4	2.4	2.4	0.6	0.0
mg mineral N	1.11	0.45	0.28	0.26	0.30	0.33	0.27
mg mineral N added in extract	1.03	0.16	0.04	0.03	0.04	0.03	0.00

Here again although decomposition of the added soluble material occurred, roughly proportional to the amount added, this was not accompanied by a concomitant production of mineral nitrogen, the high values for extracts 1 and 2 being due to the amounts initially present in the added extracts. This observation was confirmed by subsequent experiments, one of which (Expt. 3) is described below.

Experiment 3. The decomposition of aqueous soil extract

Four 25-g portions of oven-dry soil (as above) were treated as follows: Two portions were each separately extracted for ½ hour with mechanical shaking with 50 ml water, filtered at the pump and put in the respirometer (Treatments A and B, weight of moist soils = 35 g). Each of the extracts was evaporated to 10 ml. One of these was added to 25 g of the oven-dry soil (treatment C) and the other to 50 g of sand to which 0.1 g of fresh soil (as an inoculum) was added (treatment D). To the fourth 25–g portion of oven-dry soil 10 ml of water were added (treatment E). The samples were put in the respirometer for 8 days after which the amounts of carbon mineralised and the amounts of mineral nitrogen present were determined (Table 5).

TABLE 5

Treatment	A	B	C	D	E
mg C mineralised	22.8	22.8	38.4	12.0	30.0
mg NH_4-N	3.98	4.05	4.00	0.29	4.00
mg NO_3-N	0.24	0.24	4.58	2.29	2.38

The magnitudes of decomposition are C (soil and extract) > E (no extraction) > A and B (extracted) showing the decomposable nature of the soluble material. Since oven-dry soils were used any differences in production of mineral nitrogen will be reflected in the

NH_4-N data since the nitrate-producing organisms are killed. These data are, however, very uniform. The differences in the amount of NO_3-N are not due to mineralisation but to the initial leaching of nitrate from A and B compared with no leaching (E) and the addition of the leachate to C. Thus,

mg nitrate-N leached out (E—A or B) = 2.38—0.24 = 2.14.
mg nitrate-N in leachate (2.14) + that already in soil (2.38) = 4.52.
mg nitrate-N recovered in soil + extract (c) = 4.58.

Similar results were obtained in a further experiment in which aqueous extracts of both air-dried and oven-dried soils were added to air-dry soil (in which nitrifying organisms are active) and compared with the leached soil and control soil (no leaching or addition of extract).

Briefly, the following results were obtained; (a) the amount of soluble organic nitrogenous material in the oven-dry soil extract was greater than that in the air-dry soil extract; (b) decomposition of the soluble material occurred in each instance when added to air-dry soil and was roughly proportional to the amount added; (c) decomposition was less in the leached soils than the non-leached soils, and less in these than the soil plus extract. In spite of these differences in decomposition there was no evidence whatsoever of concomitant differences in nitrogen mineralisation, the magnitude of which was uniform in all instances.

Experiment 4. The rate of solution of organic material

Twenty-gram portions of oven-dried soil were covered with 50 ml water and 2 drops of toluene, shaken intermittently, and filtered after different periods. The amounts of organic nitrogen in the extracts were then determined by evaporation followed by digestion (Kjeldahl's method). The results, allowing for ammonia nitrogen extracted, are given in Table 6.

TABLE 6

Period extracted	mg org. N in filtrate	% of total (2.550)
2 hours	1.850	72.5
1 day	2.175	85.3
2 days	2.475	97.0
3 ,,	2.500	98.0
4 ,,	2.500	98.0
14 ,,	2.550	100.0

From these data the rate of solution appears to be fairly rapid.

Experiment 5. The effect of the length of the drying period on the amount of organic material subsequently extracted

Twenty-gram portions of moist soil (approx. 6% C and 0.6% N) which had been constantly moist in the incubator for 4 weeks were dried for different periods (a) in the air (b) in the oven at 100° C. The soils were then extracted with 50 ml of water with mechanical shaking for ½ hour and the extracts analysed for organic nitrogen. The results are given in Table 7.

TABLE 7

Period dried	mg organic N in extracts	
	Air-dried	Oven-dried
Nil	0.15	0.15
3 hours	—	0.35
7 hours	—	0.50
1 day	0.20	0.65
2 days	—	0.95
4 ,,	0.20	1.35
7 ,,	0.35	—
8 ,,	—	1.35
35 ,,	0.43	—

A gradual increase in the amount of material extracted is apparent on prolongation of air-drying and a rapid increase on oven-drying. No further loss of soil water occurred after the first day's air- or oven-drying.

Experiment 6. The effect of air-drying for different periods on subsequent decomposition and nitrogen mineralisation

This experiment was designed to measure the effects of different periods of drying on a range of soils containing from 1 to 7 per cent carbon. The results, when complete, will be reported in a separate paper. The data so far available are presented here to illustrate the effect of prolongation of drying on subsequent decomposition and nitrogen mineralisation.

A range of air-dry soils containing 1, 2, 3, 4, 5, 6 and 7 per cent total carbon were brought to field capacity and put in the respirometer for 14 days. After this period 35 g of the moist soil were put in the respirometer for a further 19 days and the amounts of carbon mineralised, and mineral nitrogen present, were measured. Other 35-g portions were air dried for 3, 6, 9, 12 and 15 weeks after which they were brought to field capacity and put in the respirometer for 19 days, followed by the determination of the amounts of carbon mineralised and mineral nitrogen present. The results to date are given in Table 8.

TABLE 8

% C	Weeks drying				
	0	3	6	9	12
a) mg mineral N per 100 g soil (dry basis)					
7.0	27.4	40.6	47.7	50.9	53.7
6.0	22.1	29.8	30.5	34.8	
5.0	18.5	24.4	26.1		
b) mg C mineralised per 100 g soil (dry basis)					
7.0	58.3	92.0	121.6	137.7	146.3
6.0	57.3	79.1	83.7	98.4	
5.0	55.9	72.9	86.4		

These data show that the longer the soils are dried the greater is the subsequent decomposition and nitrogen mineralisation when they are remoistened. Since the moisture content of the air-dry soils showed no significant decrease after 3 days drying the enhanced decomposition and mineralisation that occurs must be related to the persistance of the dry state. The magnitude of the drying effect on subsequent nitrogen mineralisation can best be appreciated when the N data in Table 8 are expressed as equivalent amounts of ammonium sulphate (20% N) per acre 6″ of soil (2 million lbs.), by multiplying by 100. It is then seen that after drying the 7% C soil for 3, 6, 9, and 12 weeks the amounts of nitrogen mineralised compared with no drying are equivalent to 1,320, 2,030, 2,350 and 2,630 lb sulphate of ammonia per acre. For the 6% C soil the figures are 770 lb., 840 lb. and 1,270 lb. (3, 6, and 9 weeks respectively) and for the 5% C soil 590 lb. and 760 lb. (3 and 6 weeks respectively). The increases in nitrogen mineralisation, as mentioned in the discussion, are a function of the log of the time the soil is dried, at least for the 7% C soil.

Experiment 7. Effect of different methods of drying

Thirty-gram portions of soil (approx. 6% C and 0.6% N) that had been kept constantly moist for 7 weeks in the incubator were treated as follows:

Control. Put direct in the respirometer and kept there for 9 days.

A Air dried for 3 days (22.2 g)

B Air dried for 3 days then at 100° C for 48 hours (20.6 g).

C Vacuum dried over phosphoric oxide for 3 days (20.8 g).

D As for C then dried at 100° C for 48 hours (20.7) g.

E Dried direct at 100° C for 48 hours (20.6 g).

The figures in brackets refer to the weight of the samples after these treatments. The soils were then brought to 30 g with water and put in the

respirometer for 9 days. The amounts of carbon mineralised and mineral nitrogen were then determined (see Table 9)

TABLE 9

Treatment	mg per 100 g soil (oven-dry basis)	
	C mineralised	Mineral N
A	29.1	28.05
B	128.1	39.75
C	40.8	28.52
D	116.4	37.84
E	110.7	38.16
Control	23.3	27.45

A comparison of the data for treatments B, D and E shows that the different methods of oven-drying had little effect on the subsequent magnitudes of decomposition and nitrification. Treatment C, in which the soil was dried virtually to an oven-dry state, did not differ greatly in its effect from air-drying (treatment A), and this had been noted in other experiments.

Experiment 8. The rates of nitrification and carbon mineralisation in a freshly moistened soil.

Soil (approx 6% C, 0.6% N) which had been air dry for a long time was brought to field capacity. One portion (40 g) was immediately extracted for the determination of ammonia- and nitrate-nitrogen. Other portions were put in the respirometer for the periods shown in Table 10, after which the amounts of carbon mineralised and mineral nitrogen were determined (Table 10).

TABLE 10

Days in respirometer	mg/100 g soil (air-dry basis)		C/N	mg mineralised per day	
	C mineralised	Mineral N		C	N
0	0.0	10.75	—	—	—
1	28.1	13.53	10.1	28.1	2.78
2	48.8	16.23	8.9	20.7	2.70
3	63.8	17.10	10.0	15.0	0.87
4	67.5	17.41	10.1	3.7	0.31
6	86.2	18.73	10.8	9.4 (av)	0.66 (av)
8	99.4	20.12	10.6	6.6 (av)	0.70 (av)
12	116.2	22.82	9.6	4.2 (av)	0.68 (av)
20	153.7	25.32	10.5	4.7 (av)	0.33 (av)

The amounts of nitrogen mineralised are obtained by subtracting 10.75 from the remaining values, and these subtracted values are

used to calculate the C : N ratios for the mineralisation process. The rate of carbon mineralisation falls off rapidly after the first day, and of nitrogen mineralisation after the second day. Otherwise both processes appear to follow an approximately parallel course as shown by the C : N ratios. These ratios may however be misleading particularly during the early period 1 to 3 days since it has been shown (Expts. 2 and 3) that decomposition of soluble organic material (which is particularly marked during this period) is not attended by nitrogen mineralisation. Allowing for this it would appear that the organic material involved in the nitrogen mineralisation is of lower C : N ratio than that shown.

Experiment 9. The extraction of organic material after each successive oven-drying

Thirty-five grams of oven-dry soil (approx. 6% C, 0.6% N) were extracted (mechanical shaking) for ½ hour with 150 ml water. After filtration at the pump the filtrate was evaporated to 5 ml and digested for the determination of total nitrogen (Extract No. 1). The soil was put in the oven (100° C) for 48 hours and again extracted as above. (Extract No. 2). The procedure was repeated 12 times. The amounts of organic nitrogen (total nitrogen minus mineral nitrogen) in each of the extracts are given in Table 11.

TABLE 11

Extract No.	mg org. N	Extract No.	mg org. N
1	3.35	8	2.45
2	3.10	9	2.30
3	3.23	10	2.14
4	2.63	11	1.99
5	2.80	12	1.90
6	2.45	13	1.80
7	2.63		

It will be noted that there is only a gradual decline in the amount of material in each successive extract. This should be compared with the rapid decline noted with successive extractions of a moist soil (Expt. 2). The gradual decline noted above parallels the gradual decline in the magnitude of each successive flush of decomposition following successive oven-drying and rewetting of a soil (Birch[2]).

Experiment 10. The effect of ether vapour on subsequent decomposition

Thirty-gram portions of moist soil (approx. 6% C, 0.6% N) which has been in the incubator for one month were placed in the respirometer vessels

(8-oz. sample bottles). These were then put in a vacuum desiccator, together with a beaker containing ether, and the desiccator was evacuated. The samples were removed after different periods (0.5, 1.0, 3.0 and 6.0 hours), the excess vapour removed by suction for 5 minutes, and the samples put in the respirometer. When the flush of decomposition was over (usually after 6 days) the vapour treatment was repeated and several successive decompositions were recorded. Since soils inoculated with 0.1 g fresh soil after the treatments behaved almost identically with non-inoculated soils average values are recorded to avoid duplication. With the soils exposed for 1 and 3 hours to vapour a comparison was made between those with charcoal (1.5 g) and those without. Duplicate samples (30 g), treated as above, were also extracted with 100 ml water and the extracts analysed for organic nitrogen. The data showed that the treatments in no way effected any increased solubility of organic material.

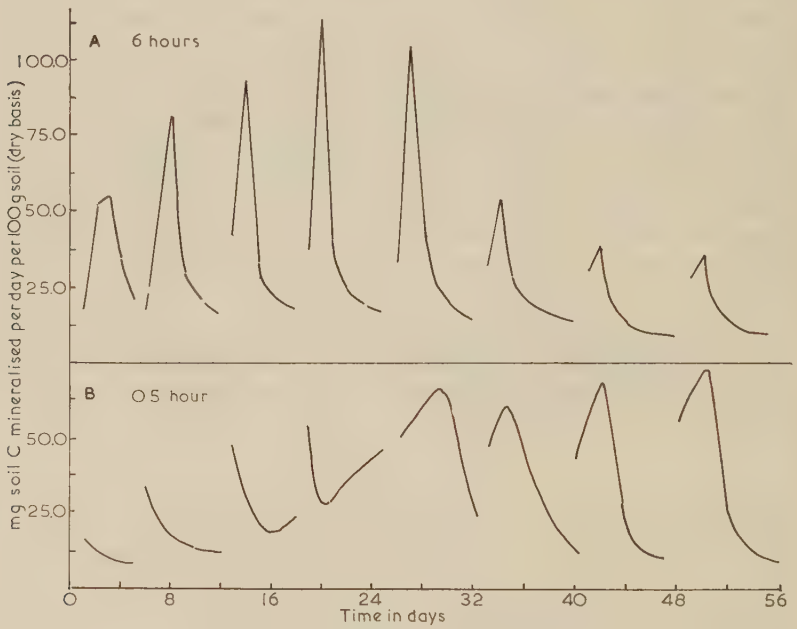


Fig. 2. Effect of successive treatments with ether vapour on subsequent decomposition.

The decomposition patterns (Fig. 2) are interesting. They reveal that successive treatments without bringing about increased solution of organic material can, nevertheless, lead to a series of flushes of decomposition, similar to those following successive air- or oven-dryings of a soil. The decomposition patterns in Fig. 2 appear to be due to the effect of the treatments on the microbial

population. With the 6-hour treatments there is a lag period of about 1 day before the maximum rate of decomposition occurs, and a sudden drop in the magnitude of this following the sixth successive treatment. This behaviour is more or less paralleled by the 3-hour treatments except that the lag period disappears after the first two treatments. Charcoal had little effect on the magnitude of decomposition. The 1-hour treatments also showed a 1-day lag period for the first two decompositions, but this subsequently disappeared and then following the sixth successive treatment the flush of decomposition disappeared altogether. Here again charcoal was ineffective in decreasing the magnitude of decomposition. The $\frac{1}{2}$-hour treatments show a gradual build-up

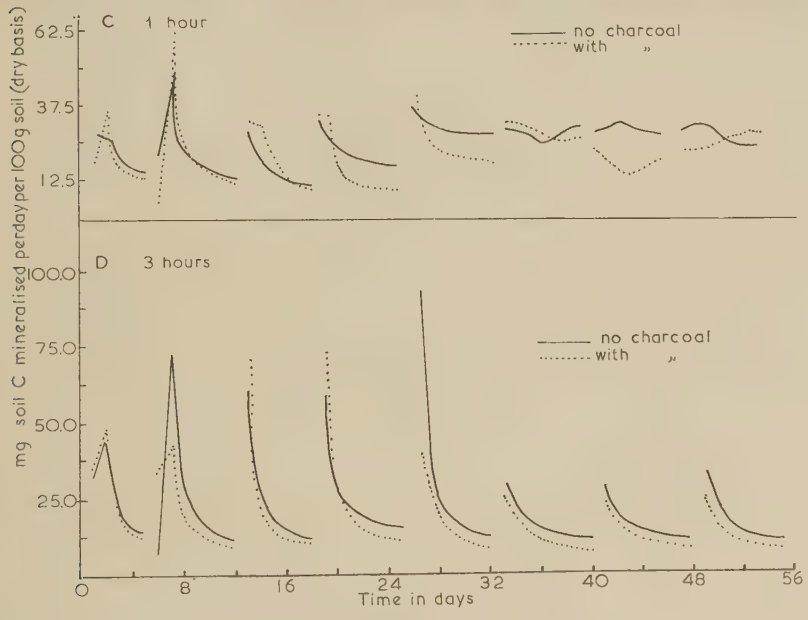


Fig. 2. (contd.) Effect of successive treatments with ether vapour on subsequent decomposition.

in the magnitude of successive decomposition with a shift in the pattern of decomposition from one in which no lag period is apparent to one where it is. This indicates a gradual change from one kind of microbial population to another with successive treatments, a change which occurs after the first 6-hour treatment. A build-up of actinomycetes may be involved (see Discussion).

It is interesting to note how sensitive the microbial population is to different periods of the same treatment. Evidently the killing of the bulk of the population is followed by a period of high activity once the adverse conditions are removed, but it is not possible to say whether this behaviour involves a specific group of micro-organisms in each instance or whether, as in Fig. 2B for example, there is possibly a gradual elimination of one or more groups to the advantage of others. The only microbial identification carried out in this experiment (which was designed primarily to test for the activity of a newly developing population rather than the specific micro-organisms involved) was on the soils treated for 6 hours with ether vapour in which a mycelial-like growth was evident. This was identified by Dr. Millbank as actinomycetes.

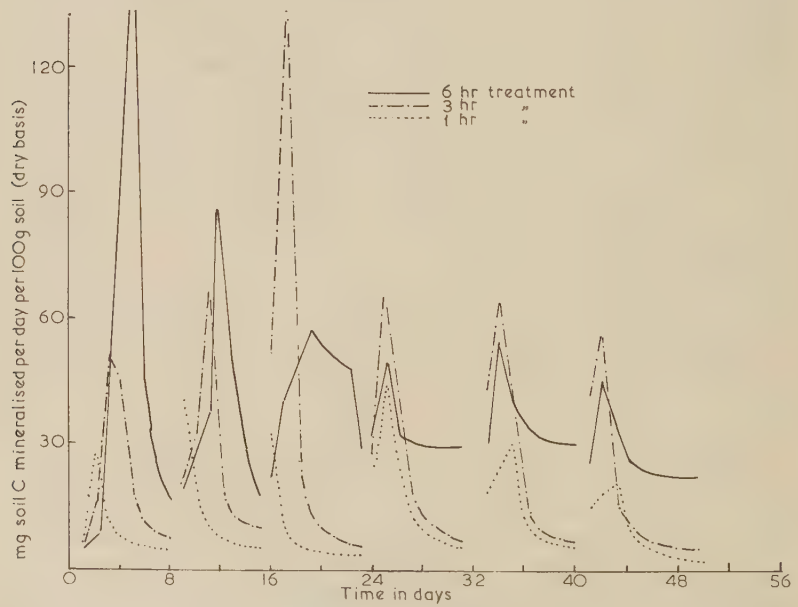


Fig. 3. Effect of successive treatments with chloroform vapour of subsequent decomposition.

Experiment 11. The effects of chloroform vapour and formaldehyde vapour on decomposition

Forty-gram portions of moist soil (approx. 6% C and 0.6% N) which had been in the incubator for 5 days were exposed for 1, 3, and 6 hours in a vacuum to chloroform vapour in one instance and formaldehyde vapour in

the other, using the same technique as that employed in Expt. 10. When the flush of decomposition was over the treatments were repeated, altogether six times. The soils were then extracted for ammonia- and nitrate-nitrogen. The treatments, as in Experiment 10, had no effect on the amount of extractable water-soluble organic material. A control was included in the experiment.

The patterns of decomposition are shown in Figs. 3 and 4 while Table 12 shows the amounts of carbon mineralised and mineral nitrogen present after the six treatments.

TABLE 12

Treatment and period	per 100 g soil (oven-dry basis)			
	mg C mineralised	mg NH_4-N	mg NO_3-N	NH_4-N + NO_3-N
Chloroform vapour				
1 hour	253	106	63	169
3 hours	441	61	16	77
6 hours	742	5	6	11
Formaldehyde vapour				
1 hour	209	39	100	139
3 hours	264	84	67	151
6 hours	439	70	40	110
Control *	172	5	138	143
Blank **	—	5	87	92

* Control refers to soil in the respirometer for periods equal to the treated soils.
** The blank refers to the soil at the start of the treatments.

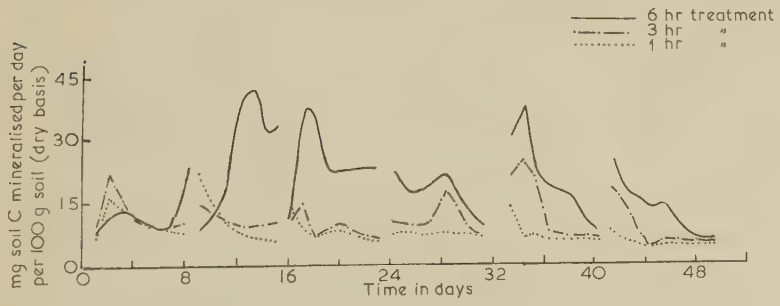


Fig. 4. Effect of successive treatments with formaldehyde vapour on subsequent decomposition.

The patterns of decomposition are interesting. While the decomposition peak with the 6-hour chloroform-treated samples falls

rapidly with successive treatments, the equilibrium rate of decomposition, after the flush is over, increases and then shows a gradual decline. With the 3-hour chloroform treatments the highest peak decomposition occurs after the third treatment and then declines, but no high equilibrium rate occurs. The formaldehyde-treated soils show similar effects but of much smaller magnitude.

From the nitrogen data it appears that the nitrifying organisms have been affected to various extents. The 3-hour and 6-hour chloroform treatments are considerably lower in mineral nitrogen than the control and the blank. It appears that ammonfication and nitrification processes had virtually ceased under these conditions (particularly with the 6-hour treatment) and that the nitrate originally present in the soil has been utilised by the micro-organisms.

Experiment 12. The effect of successive heat treatments (dry and moist) for different periods on the successive magnitudes of decomposition

Thirty-gram samples of moist soil (approx. 6% C, 0.6% N) which had been in the incubator, constantly moist, for 2 weeks were put in the oven at 100° C for 7 hours, 1, 2, 4, and 12 days. After these periods the soils were brought to field capacity and put in the respirometer. After 9 days the amount of carbon mineralised was determined and the heat and remoistening treatments repeated, 11 times in all (7 times for the 12-day treatment). Similarly 2 soils were successively heated for 3 hours and 24 hours at 100° C without water loss, and decomposition measured after they had cooled.

In Figs. 5 and 6 the magnitudes of each successive decomposition for the 9-day period in the respirometer (6 days for the soils heated without drying) are shown, rather than each successive flush of decomposition individually. In this way the graphs show the decline in magnitude of each successive flush of decomposition and not the decline in the rate of decomposition for a single flush.

With the 7-hour oven-dried soil the magnitude of each successive flush of decomposition declined rapidly and after the third successive treatment reached a steady value. With the 1-, 2-, and 4-day oven treatments the magnitudes of decomposition were greater and the decline in each extended over a greater number of successive treatments. With the 12-day treatment the magnitudes of decomposition were high and their rate of decline with successive treatments much less. When however, the soils heated for the shorter periods (7 and 24 hours) were heated for 48 hours or longer at the termination of the experiment a recovery occurred in the magnitude of

decomposition on rewetting. Similar results were obtained with the soils heated moist for different periods (Fig. 6).

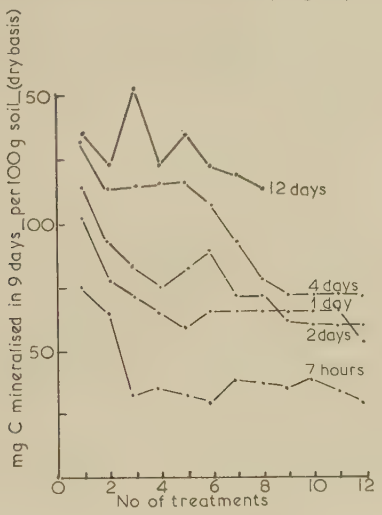


Fig. 5. Magnitudes of decomposition after each successive oven-drying for different periods.

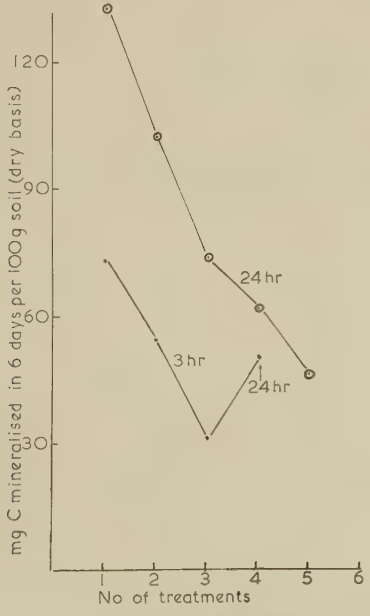


Fig. 6. Magnitudes of decomposition after each successive heating (no drying) for different periods.

Since no further perceptible loss of water occurs with further heating (100° C) beyond the 24-hour period the enhanced effect of the 4- and 12-day heating must be due to slow physical changes, and this factor apparently operates in Expt. 6 and also under conditions of moist heat.

DISCUSSION

Experimental evidence has been presented to indicate (a) that the short period of rapid decomposition following the moistening of a dry soil is primarily due to microbial decomposition of soluble organic material, (b) that although enhanced nitrogen mineralisation also occurs this is not due to the decomposition of the soluble material and (c) that a short period of rapid decomposition also follows treatments that do not affect the amount of organic material going into solution. These three observations will be discussed in turn.

Humus decomposition

When a dry soil is moistened organic material goes into solution and is rapidly decomposed. The magnitude of this flush of decomposition depends on the percentage carbon in the soil (Birch [2]) and the length of the drying period without this necessarily involving a greater degree of drying (Expt. 6). The behaviour is repetitive with successive dryings and rewettings and also occurs under field conditions (Birch [2]). The magnitude of the effect is considerably enhanced when the soil is heated, whether moist or dry. It has also been noted (Birch [2]) that the drying effect on humus was little affected by the soil type with which the humus was associated and even occurred in a humus–sand mixture. The drying effect must therefore largely depend on changes in the organic fraction, and the experimental data indicate that organic colloids are largely involved.

From Experiment 7 it is unlikely that the drying effect involves movement of soluble material from within cellular material to the outside, where it is decomposed after wetting. Chemical changes could occur, but Lebedjantev [10] has shown that oxidation changes are not involved and the author has found that successive dryings (or heatings) and rewettings have no cumulative effect as regards subsequent decomposition (Birch [2]). It appears that

the state of the organic colloids after drying is the main factor governing subsequent decomposition and nitrogen mineralisation with progressive changes taking place as the dry colloids age, these changes being accelerated by heat. Boutaric and Thevenet [4] have shown that solutions of humus colloids decline in viscosity with time and that the decline is hastened at higher temperatures. This behaviour closely parallels that reported in Expt. 5 on the effect of time and temperature on the amount of material subsequently going into solution, indicating that colloidal changes are also involved here.

A certain degree of drying (approximately to the air-dry state) is required for the drying effect to occur. At this stage residual shrinkage in a soil occurs. According to Haines [8] this shrinkage involves the organic fraction of the soil. He also considers it possible that transition from the sol to the gel form takes place at this stage. Thereafter with prolongation of the dry state, physical changes occur in the gel on ageing. These changes appear, from Expt. 6, to conform to a basic principle. Thus, for the 7% C soil the magnitudes of decomposition and nitrogen mineralisation on moistening after drying for different periods is a direct function of the logarithm of the time the soils were dried. There is no direct experimental evidence to show what actual changes take place in the gel on ageing. From the experimental data it appears that drying leads to an increased exposure of organic surface, which would account for the extra amount of organic material that can be extracted from a dry soil compared with a moist one. Comber [6] contends that the first effects of heating a soil will be dehydration, shrinkage and cracking of the gel. Fragmentation of the gel on drying or heating would certainly lead to the exposure of a greater surface area. The behaviour of swelling gels on drying and remoistening is reversible, so that after moistening the soil the gel fragments would swell and cohere with consequently reduced surface. Alternatively, during ageing of the gel, the micellae and intervening capillary spaces may become increasingly coarser, as decribed by Freundlich [7] in discussing the behaviour of silica gel. Coarsening of the capillary spaces would lead to a greater area of contact with the added water. Reversibility of this reaction would involve swelling with closing of the capillary spaces, reducing the available surface to that characteristic of prolonged moist conditions.

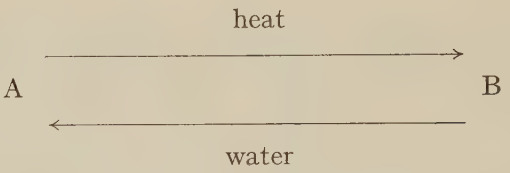
Further evidence that the drying effect mainly involves organic material rather than changes in the soil containing it is furnished by the following observations. When, after the flush of decomposition is over, a moist soil is ground in the mortar and returned to the respirometer no enhanced decomposition occurs. Such treatment would hardly effect the colloidal state, but if the drying effect were due to changes during aggregation (on drying) or disaggregation (on wetting) of the soil, leading to the exposure of organic surfaces hitherto protected by contact between particles, one would expect reorientation of the surfaces, through grinding, to have a similar effect and lead to enhanced decomposition. Secondly, when a solution of gelatine was added to two soils rapid decomposition occurred. Subsequent successive dryings (air or oven) and rewettings then led to successive flushes of decomposition greater than those obtained prior to the addition of the gelatine. Similar behaviour follows the addition of plant material to soils and in each instance it appears that the decomposition products of the added material then function in the drying effect. In the gelatine experiment the soils used were very different, but the effect following the addition of gelatine were practically the same. This also applies to the experiments involving the addition of plant material (Birch [2]).

It was earlier postulated (Birch [2]) from the evidence then available that enhanced solution of organic material was not the factor involved in the flush of decomposition that follows the moistening of a dry soil. Thus, soils saturated with different bases furnished very different amounts of water-soluble organic material, after air- or oven-drying, but decomposed to an approximately similar extent on moistening. Also, decomposition of an extracted soil was only slightly less than with those to which the extract (unconcentrated and concentrated) was added. In the latter experiment the extraction conditions were not as vigorous as in Expt. 2, and this would account for the initial failure to obtain any appreciable effect following extraction. It is more difficult to explain the results obtained with the soils saturated with different bases unless the organic material going into solution, particularly following peptisation in the Na^+- and K^+-treated soils, is not identical with that going into solution when a normal soil is moistened, and represents part of a non-decomposable humus fraction. The

hypothesis advanced by Chase and Gray [5] to account for the two superimposed reaction rates involved in the decomposition process following the moistening of a dry soil (one of these involving rapid decomposition of soluble material) is therefore probably correct and the objections raised to this (Birch [2]) no longer valid. The first-order reactions described (Birch [2]) likewise did not conform strictly to a simple first-order reaction and it is evident that concomitant with the rapid decomposition of the soluble material, there could be a fall-off in microbial activity at the surface (see Expts. 10 and 11) or in the area of surface exposed. Each of these changes could also conform to a first-order reaction.

The mechanism outlined above satisfactorily explains most of the observations made in the experiments described in this and the previous publication (Birch [2]). Since the state in which the organic colloid or gel is left as a result of drying or heating is the main factor governing subsequent decomposition this would explain (a) why a single drying and rewetting is as effective as a succession of such treatments (without immediate decomposition) on the subsequent magnitude of decomposition when this is allowed to occur (Birch [2]) and (b) why this magnitude is also independent of the time after rewetting that a soil is again dried (Birch [2]). Basically the colloids are left in the same physical state after drying regardless, within limits, of the period that the soil was moist prior to this or the number of intermediate wettings and dryings it has received. The qualification "within limits" refers to the fact that as the organic material involved in the drying effect is gradually depleted, as a result of successive decompositions or prolonged moist conditions, the magnitude of the drying effect will also decline (Birch [2]). The effect of heating without drying (Expt. 12) is less easy to explain. If, however, the decline in viscosity of humus colloids with time and temperature mentioned earlier is due to decreased floculation, resulting in a smaller amount of occluded water (See Baver and Hall [1]), then enhanced decomposition could again be a result of increased surface.

Fig. 5, Expt. 12 shows that only with the longer heat treatments can a series of flushes of approximately equal magnitude be obtained. Presumably the milder the treatments the smaller the changes in the colloids and the more they fall short of state B in the system



where A and B denote the states of the colloids under prolonged moist and hot dry conditions respectively. After wetting a mildly treated soil it will return more completely to state A than the strongly treated soil, and consequently on again drying the changes will not proceed so far in the direction of B. Successive mild treatments and rewettings will have a progressive effect in favour of A until finally the mild treatment no longer brings about any marked changes in the direction of B. By increasing the severity of the treatment at this stage the changes towards B are re-established and a flush of decomposition again follows.

With regard to humus decomposition under field conditions, at the start of the rains there will be rapid solution and decomposition of soluble organic material without concomitant nitrogen mineralisation, the site of decomposition in the profile depending on the severity of the rains and the drainage of the soil. At the same time decomposition of organic material at the organic surface also takes place. This continues as long as the soil is moist and also shows a decline in the rate of decomposition due possibly to a decline in microbial activity or a decrease in exposed surface as the colloids come to equilibrium. This surface decomposition involves nitrogen mineralisation.

Soil nitrogen mineralisation

Experiments 2 and 3 show that nitrogen mineralisation of humus is a surface phenomenon and will therefore depend on the surface area and conditions at the surface. Generally the greater the total nitrogen or organic carbon content of the soil the greater the amount of carbon and nitrogen mineralised. For a given soil mineralisation of carbon and nitrogen is enhanced both by drying (or heating) and prolongation of these states (Expt. 6). The decline in the rate of nitrogen mineralisation, after moistening, is rapid (Expt. 8). Nitrogen mineralisation depends on the prior decomposition of organic material so the effect of drying on subsequent nitrogen mineralisation could be due to the increased organic surface exposed,

as already described. The rapid decline in the rate of nitrogen mineralisation indicates a rapid reduction in the area of the surface where nitrogen mineralisation occurs possibly as a result of swelling of the organic colloids. Decreased microbial activity as the microbial population ages can also be involved in this decline.

It appears from Expt. 8 that the material involved in the nitrogen mineralisation has a C/N ratio somewhat less than 10. Since, on moistening a dry soil two decomposition processes take place, namely decomposition of soluble material involving liberation of carbon dioxide without any mineralisation of nitrogen and surface decomposition involving both carbon and nitrogen mineralisation many of the C/N ratios reported in the literature, in relation to the actual material involved in nitrogen mineralisation, are probably too high since these values include carbon dioxide derived from non-nitrifiable material.

The effect of drying for different periods on the amounts of carbon and nitrogen subsequently mineralised seems to conform to a definite law. Thus, for the 7% C soil (Expt. 6) a very significant linear relationship was found between the log of the number of weeks (X) that the soil was dried and the amount of nitrogen mineralised as a result of this during 19 days after moistening (Y_n).

$$Y_n = 21.63 \log X + 3.06 \ (r^2 = 0.9976) \ \ldots\ldots\ (1)$$

The corresponding equation for carbon mineralisation was found to be

$$Y_c = 91.31 \log X - 9.94 \ (r^2 = 0.9963) \ \ldots\ldots\ (2)$$

Y_n and Y_c = mg/100 g soil (dry basis) and since they are obtained by subtracting col. 2 from cols. 3, 4, 5, and 6 (Table 8) they refer to the extra amounts of carbon and nitrogen mineralised as a result of drying.

Further discussion of Expt. 6 must await its completion. Possibly the results described above refer only to highly organic soils, but present indications are that similar relationships will be found for soils of lower carbon content. If so these simple relationships should find application in field studies involving humus decomposition and soil nitrogen (particularly where well-defined wet and dry seasons occur) and in irrigation if the dry period can to some extent be controlled. Until the data for this experiment are complete

it may tentatively be concluded that following the moistening of a dry soil a short period of relatively rapid nitrogen mineralisation takes place the magnitude of which is directly proportional to the log of the period of drying and the organic content of the soil.

Partial sterilisation

The main conclusion derived from Expts. 10 and 11 is that marked and successive flushes of decomposition can occur in the absence of any enhanced solution of organic material such as occurs after drying. The flush of decomposition appears to be due to the activity of a freshly developing microbial population this activity falling off as the population ages and as conditions at the organic surface deteriorate due to crowding of the bacterial population (see Magoon and Brunstetter [10]). No attempt was made to follow changes in the numbers and kind of micro-organisms in these experiments apart from the identification of actinomycetes on the soils treated with ether vapour for 6 hours. This observation may be significant since Vandecaveye and Katznelson [13] reported that while water-soluble carbon and nitrogen compounds decomposed very rapidly particularly when bacteria and fungi were most active, loss of sulphuric acid-soluble carbon and nitrogen compounds was greatest in soils with the largest number of actinomycetes, suggesting that these substances constitute a more suitable food for actinomycetes than for bacteria and fungi. This would indicate recurrent cycles of surface decomposition in which actinomycetes are involved, though this does not preclude the possibility that bacteria and fungi are also active.

A part of each successive flush of decomposition may be due to the utilisation by the newly developing population of micro-organisms killed by the vapour treatments. Jensen [9] reports that dried cell substance of various micro-organisms is rapidly decomposed in soils with production of nitrate, and Steinhaus and Birkeland [11] have similarly reported the cannibalisation of dead cells by living ones. Since, however, half or more of the carbon in the dead material is likely to be lost as carbon dioxide during decomposition succeeding flushes of approximately equal magnitudes could only occur if the viable population was built up at the expense of the organic material in the soil.

Since soil drying and heating are also forms of partial sterilisation

there is reason to believe that microbial behaviour similar to that described above also follows these treatments. Thus, on moistening a dry soil there will be, in addition to the rapid decomposition of soluble material a period of relatively high microbial activity as the population develops, with marked mineralisation of carbon and nitrogen, this activity falling off as the population ages and adverse surface conditions develop. This behaviour is demonstrated in Fig. 1 which shows both the rapid decline in decomposition of soluble material, and a concomitant decline in decomposition in soils from which the soluble material was removed by activated charcoal.

An interesting feature revealed in this work is that in relating yield and rainfall account must also be taken of the length of the dry period prior to the rains since this, for a given soil, determines the amount of mineral nitrogen produced at the start of the rains. In countries with well-defined wet and dry seasons, yield differences following different, but adequate, amounts of rainfall may be less a function of rainfall than climatic conditions prior to the rains which govern the length of the dry period.

SUMMARY

Evidence is presented to show that the longer a soil is kept in an air-dry state the greater is the amount of water-soluble organic material that can be extracted from it even though the soil does not become drier. This effect is greatly enhanced with dry soils kept at 100° C. Soils kept air-dry for increasingly long periods also showed increasing magnitudes of carbon and nitrogen mineralisation on moistening which appear from the data so far available to be a linear function of the log of the period that the soil was dried.

The organic material going into solution on moistening a dry soil rapidly decomposes, but without concomitant mineralisation of nitrogen. This decomposition partly accounts for the flush of decomposition that follows the moistening of a dry soil. The brief period of enhanced nitrogen mineralisation that simultaneously occurs is considered to involve surface decomposition of organic material. The drying effect is shown largely to involve changes in the organic material rather than in the soil itself, and it is postulated that drying brings about changes in the organic gels that continue during ageing and are enhanced by heat. The changes visualised are fragmentation or increased porosity of the gel on drying and heating, each of these leading to increased surface area. The changes are considered to be reversible on moistening. Such behaviour would account not only for the increased

amount of organic material that can be extracted from a dry soil compared with a moist one, but also for the decline in the rate of nitrification, after moistening, consequent upon the swelling and cohesion of the gel with decreased surface area.

It is also possible that the high rate of decomposition and nitrogen mineralisation following the moistening of a dry soil, and the rapid decline in this rate, is due to the high microbial activity associated with the youthful phase of a developing microbial population, this activity declining as the microbial population ages. Evidence for such behaviour is presented in experiments with moist soils treated with organic vapours. Successive treatments resulted in successive flushes of decomposition without however involving any enhanced solution of organic material such as occurs after drying. The high activity, which rapidly declines, indicates a decline in microbial activity rather than in the amount of substrate, since this is adequate for the occurrence of several successive flushes of decomposition.

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COMPETITIVE ADAPTATION OF THE CATION EXCHANGE CAPACITY OF ROOTS

by L. K. WIERSUM and K. BAKEMA

Institute for Soil Fertility, Groningen, Netherlands

INTRODUCTION

The differences in the ratio divalent ions/ monovalent ions between plants of one species or between plants of different species, has often been related to the variation in cation-exchange capacity (C.E.C.) of the roots [2 6 7 15 17 18]. The explanation of the difference is based on the Donnan principle, which according to the relation *

$$\frac{\text{C.E.C.}_1}{\text{C.E.C.}_2} = \left(\frac{Ca_1}{Ca_2}\right)^2 = \frac{K_2}{K_1}$$

shows that plants with a higher C.E.C. of their roots will absorb relatively more Ca^{++} than K^+ from the same dilute solution. A low C.E.C. of the roots would lead to a relative preferential uptake of K^+.

Although adsorption is only the first step in a sequence of processes leading to accumulation of ions in the plant, the established relationship between C.E.C. and the relative amounts of mono- and divalent ions absorbed by the plant, point to its regulative influence.

Since the plant usually shows a great adaptibility in all its functions, it is to be expected that a characteristic which can govern a certain process would show variations. Indeed, it has already been demonstrated that enhanced nitrogen supply may result in an increase of the C.E.C., while induced metabolic dis-

* Indices 1 and 2 refer to species (or plants) no. 1 and 2. *Ca* resp. *K* = amount of Ca resp. K ions adsorbed.

turbances may also give rise to differences [3 4 10 13 16 20]. In our own experiments indications of a temperature effect were found.

In the soil the roots must compete with the soil colloids for ions [14]. Hence an experiment was devised to investigate the possibility that the root may counteract the competitive action of the soil colloids by variations in its C.E.C.

METHODS

Seeds of several plant species were germinated on moist filter paper or sand. As soon as more than one root was visible or root branching had started the seedlings were transferred to the experimental vessels.

The experiment was conducted in glass jars of about one litre capacity. These jars contained a vertical partition reaching to about $\frac{3}{4}$ of the height. On one side of the partition the jar was filled with coarse sand, to which a cation-exchange resin (Amberlite IR 120), previously saturated with a mixture of cations against a Hoagland nutrient solution, had been added. The other side was filled with sand mixed with an anion-exchange resin (Amberlite IRA 400), saturated with an anion mixture by contact with the same nutrient solution. In a few cases the sand was mixed with resin saturated

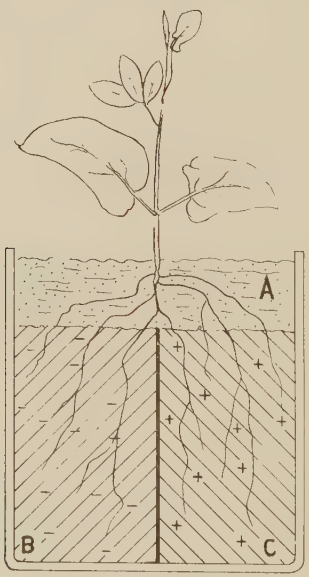

Fig. 1. Diagram of the experiment. A. Toplayer of sand. B. Compartment filled with sand mixed with an anion-loaded exchange resin. C.Compartment filled with sand mixed with a cation-loaded exchange resin.

with a single ion. The mixtures contained ab. 7 ml resin in 100 g dry sand. The top part of the jar was then filled with coarse sand, in which the young seedling was planted (Fig. 1).

After a period of a few weeks growth, when roots were clearly visible in the lower part of the jar, the roots were collected. The toplayer of sand and the upper part of the root system in it was first removed. Then the roots contained in the two compartments could be washed out of the sand–resin mixture separately. According to the amount of roots extracted, samples of one or more split root systems were used for determination of the C.E.C.

The method of determining root C.E.C. somewhat resembled that of Helmy and Elgabaly [9].

About 2 g fresh roots are rinsed in 100 ml HCl (pH 2) for exactly 40 sec. This is repeated a second time in a fresh acid solution. The roots are then rinsed with demineralized water till free from chloride. After this treatment the roots are put into a solution of KCl, 1 *N* and pH 8, for 2 minutes. The KCl solution, which has become acid by exchange against root-H^+, is titrated to a pH of 8.0 with KOH 0.03 *N*. The amount of KOH added is a measure for the amount of liberated H^+, which is itself a measure for the root C.E.C. The C.E.C. is expressed in me per 100 g dry material. As the two parts of one root system are compared it is not necessary to express root C.E.C. on a surface basis, although this method might be preferred when comparing different species [1 19].

RESULTS AND DISCUSSION

A summary of the results obtained in the experiments is given in Table 1. Most of the data are based on duplicate analyses, which generally show only minor variations.

With the exception of the experiments conducted with broad bean, where the large stock of reserves in the seed may have influenced the results, all the plant species tested showed a higher C.E.C. in the medium containing the cations.

A high C.E.C. in the cation-containing medium, where most ions are in the adsorbed state on the resin, would be of advantage for the plant in competing for ions with the resin. In the anion-containing sand–resin mixture a high C.E.C. might on the contrary even be considered as disadvantageous as on account of the Donnan equilibrium entrance of anions into "the apparent free space" might be restricted.

The conclusion can thus be drawn that in the experiments conducted the occurrence of a competitive adaptation of the root C.E.C. in relation to its surroundings has been demonstrated.

The fact that an adaptive variability of root C.E.C. occurs supports the interpretation that the level of root C.E.C. has a regulating influence on the intake of the amounts of monovalent and divalent ions absorbed. This regulating influence may be related to the effect of the Donnan equilibria, but it has also been shown that the root possesses selective adsorptive capacities relating to chemical constituents [2 5 11 12].

TABLE 1

Comparison of the C.E.C. (expressed as me per 100 g dry root weight) of two parts of split root systems of a single plant in two different media

Plant species	Root medium	
	Amberlite + cation mixture	Amberlite + anion mixture
Wheat (*Triticum vulgare*) . . .	2.2	2.0
Bean (*Phaseolus vulgaris*) . . .	7.6	5.4
Pea (*Pisum sativum*)	10.4	8.8
Cress (*Lepidium sativum*) . . .	20.3	16.2
Broad bean (*Vicia faba*). . . .	7.3	10.4
	Amberlite + Ca^{++}	Amberlite + $H_2PO_4^-$
Broad bean (*Vicia faba*). . . .	9.2	9.7
Tomato (*Solanum lycopersicum*)	4.7	4.5
	Amberlite + Mg^{++}	Amberlite + SO_4^{--}
Turnip (*Brassica rapa*)	10.7	10.2

Nevertheless the regulative influence of the root C.E.C. is not the only factor governing ion uptake. In the "active phase" of ion absorption, which is supposed to be performed by "carriers" with an expenditure of energy released in metabolism, indications have been obtained of very specific selectivities for separate ions [8].

The role of root C.E.C. in influencing the relative amount of mono- and divalent ions absorbed may best be understood as governing the ionic composition in the "apparent free space" and thus regulating the supply of ions to the carrier system.

SUMMARY

The capability of root C.E.C. in exerting a regulative influence on the relative amount of mono- and divalent cations absorbed is underlined by demonstrating an adaptivity of this characteristic.

It is shown that root C.E.C. is raised in a medium where it must compete for cations, while it is somewhat lower in a medium where only anions are available. This adaptive variation was demonstrated on the roots of single plants of several species using split root-systems.

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